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PRINCIPLES OF RESPIRATORY RHYTHM GENERATION IN MAMMALS. S1 1

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Respiratory movements are controlled by one of the most active networks in the brain of mammals. The primary rhythm generator, the respiratory center, was localized recently in the bilateral pre-Botzinger Complex, a brainstem area localized just caudal to the Nucl. retrofacialis. The center remains functional even after *in vitro* isolation in a brainstem slice. The center contains 5-6 classes of neurons, i.e., pre-inspiratory, early-inspiratory, throughout-inspiratory, post-inspiratory and expiratory neurons, defined by ongoing discharge activities. As there is no fundamental difference between pre-inspiratory and early-inspiratory activity patterns, one could argue that they constitute a single population of neurons. Rhythmic oscillations seem to start by interaction between a pair of two reciprocally connected groups of neurons, i.e., pre-inspiratory/early-inspiratory and post-inspiratory neurons.

Various mechanisms seem to cooperate during the process of rhythm generation. All types of respiratory neurons have rebound depolarization induced by low voltage-activated Ca^{2+}-currents and, in addition, endogenous bursting capacities when they are depolarized so that intermediate voltage-activated Ca^{2+} and Na^{+}-conductances are activated. Under normal *in vivo* conditions, the origin of the underlying depolarization might primarily come from the reticular formation. Synaptic transmission at the onset of periodic activity might primarily remain subthreshold, but is then amplified by endogenous bursting characteristics of homologous neurons within the network. In the *in vitro* isolated network, synaptic inhibition does not seem to be essential for maintaining rhythmic activity in a limited number of neurons. However, synaptic inhibition is essential for off-switching phase activities and stabilization of the rhythm *in vivo*. When synaptic inhibition fails under *in vivo* conditions, the rhythm comes to rest. Such inspiratory apneusis is one of the most frequent life-threatening disturbances observed in clinical practise. The respiratory rhythm is one of the most stable in our organism. One of the reasons for such high stability may be permanent adjustment of intracellular second messenger systems which are up- and downregulated by neuromodulators.

S1 2 CENTRAL MECHANISMS IN UPPER AIRWAY REFLEXES. S. González-Barón, J. P. Lara and M. S. Dawid-Milner. Departamento de Fisiología, Facultad de Medicina. Universidad de Málaga, Málaga (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 14, 1997.

Electrophysiological and histochemical studies of the central mechanisms involved in the integration of respiratory and cardiovascular reflexes have been analysed in the cat. Special interest has been put in two respiratory reflex responses evoked from the stimulation of the upper airways: the cough reflex and the apnoeic laryngeal reflex. These responses provide a suitable model for studying respiratory and cardiovascular regulation during the integration of non-respiratory and respiratory behaviours.

During cough elicited by mechanical stimulation of tracheal mucosa, an increase in linear velocities of inspiratory flows and mainly expiratory flows was observed. These effects are produced together with an increase in expiratory pleural pressure, bronchoconstriction and a decrease in larynx resistance due to a wide opening of the larynx. Laryngeal resistance, which is always proportional to the laryngeal diameter, was measured with the method of the larynx isolated *in situ*. The response of laryngeal dilatation during the cough burst does not coincide with that of the classical description of cough. The recording of respiratory nerves and of the activity of respiratory neurons located within the nucleus ambiguus (NA) in the spontaneously breathing decerebrate cat shows that the inspiratory discharge of inspiratory laryngeal motoneurons (ILMN) was changed abruptly to a phasic inspiratory-expiratory tonic discharge during the cough burst. This coincided with a sustained opening of the larynx. The other non laryngeal respiratory neurons recorded in the vicinity of ILMN within the NA, responded with a sudden increase in frequency, but their pattern of discharge remained unaltered.

During the apnoeic reflex elicited by mechanical stimulation of laryngeal mucosa (glottic or supraglottic) an expiratory apnoea with a partial or a complete glottic closure was observed. These effects are produced together with a small tonic discharge of ILMNs during the respiratory cycle, while expiratory laryngeal motoneurons (ELMN) presented variable responses. Most of ELMN responded with an intense tonic discharge lasting the glottic closure, while some remained silent. The other non laryngeal inspiratory neurons recorded in the vicinity remained also silent.

The increase of respiratory resistance during bronchoconstriction or mechanical reduction of tracheal diameter produces a decrease in the intensity of the cough reflex or the apnoeic reflex. During tracheal stimulation the inspiratory discharge of ILMN changed to a phasic inspiratory-expiratory tonic discharge. The tonic discharge coincided with a decrease in laryngeal resistance even if the cough burst was abolished.

This study suggests that laryngeal motoneurons are driven by powerful inputs from afferents of tracheal and laryngeal mucosa irritant receptors. The integration of these inputs produces different respiratory reflex responses depending on the location of the receptors in the laryngeal and tracheal regions. It has also provided an explanation for the observation that in the cat mechanical stimulation on tracheal mucosa produces a decrease of laryngeal resistance during the cough burst.

This work was supported by a grant of the DGICYT PB94-1472.

SUPRAMEDULLARY MECHANISMS IN RESPIRATORY CONTROL. M. S. Dawid-Milner, J. P. Lara, K. M. Spyer* and S. González-Barón. Departamento de Fisiología, Facultad de Medicina, Málaga (Spain) and *Department of Physiology, Royal Free Hospital School of Medicine and University College London, London (UK). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 15, 1997. S1 3

The basic respiratory rhythm is generated by the activity of neurons located in medulla oblongata while supramedullary neurons act to modulate the pattern of respiration. In previous studies we have shown that in the rat, a pontine region, the parabrachial complex, modulates respiration with two different patterns. Neurons in the medial region of the parabrachial nucleus and Kölliker Fuse nucleus are involved in the facilitation of expiration while neurons in the lateral region of the parabrachial nucleus facilitate inspiratory activity (Lara *et al.* 1994). The Kolliker Fuse nucleus and the parabrachial nucleus are connected with the A5 group of catecholamine-containing neurons of the caudal ventrolateral pons. These connections with regions modulating the cardiovascular system are indicative of a role for the A5 region in their control of cardiovascular activity, although its specific role in cardio-respiratory function has yet to be revealed.

In order to assess the importance of the A5 region in modulating cardio-respiratory activity, electric current (10-40 μA, 0.4 ms pulses, 50 Hz for 5 s) or microinjections of glutamate (20-30 nl, 100 mM, pH 7.4 ± 0.1) were used to stimulate discrete zones within this region in the anaesthetized rat (pentobarbitone, 60 mg kg^{-1}, i.p.). The stimulation of the A5 region produced an expiratory facilitatory response. This consisted of a decrease in respiratory rate ($p < 0.01$, electrical stimulation, $p < 0.05$, glutamate) due to an increase in expiratory time ($p < 0.05$ in both cases) as measured by observing phrenic nerve activity. No changes were observed in inspiratory time. At all sites where electrical stimulation and glutamate injection had evoked a respiratory response, electrical stimulation evoked a concomitant increase in blood pressure ($p < 0.001$) and heart rate ($p < 0.05$).

Glutamate injection evoked a pressor response in 21 out of 30 animals ($p < 0.05$). In 8 animals, a rise in blood pressure was elicited that was followed by a fall in blood pressure; and in 1 animal, a depressor response was observed.

The expiratory facilitatory response was not evoked as a consequence of the rise of blood pressure since it was still present after the administration of guanethidine (10 mg kg^{-1}, i.v.), which abolished the rise in blood pressure.

As glutamate is believed to excite perikarya rather than axons of passage these data indicate that expiratory facilitatory responses and the accompanying cardiovascular changes are the consequence of activating neurons within the A5 region. The possible interactions between the A5 region and the medullary respiratory complex in eliciting these changes will be discussed. This work was supported by a grant of the DGICYT PB94-1472.

J. P. Lara, M. J. Parkes, L. Silva-Carvalho, P. Izzo, M. S. Dawid-Milner and K. M. Spyer. Cardiovascular and respiratory effects of stimulation of cell bodies of the parabrachial nuclei in the anaesthetized rat. *J. Physiol.*, (1994), 477, 321-329.

M. S. Dawid-Milner, J. P. Lara, I. Rocha and S. González-Barón. Respiratory and circulatory responses to electrical and chemical stimulation in the A5 region of the anaesthetized rat. *J. Physiol.*, (1994), 481, 10P.

S1 4 OXYGEN SENSING BY ION CHANNELS AND CHEMOTRANSDUCTION IN CAROTID BODY GLOMUS CELLS. J. López-Barneo. Departamento de Fisiología Médica y Biofísica, Fac. Med. Univ. Sevilla (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 16, 1997.

Chemosensory transduction by the carotid body is a well characterized O_2-dependent process. Carotid body (glomus) cells are capable of sensing reductions in arterial O_2 tension (PO_2) and to stimulate the brainstem respiratory centers to produce hyperventilation. Electrophysiological work has shown that the chemoreceptive properties of glomus cells are based upon the presence of O_2-sensitive K^+ channels whose activity is inhibited in response to lowering PO_2 (see for a review López-Barneo *et al.*, 1993). Hypoxia results in the enhancement of cellular excitability and leads to Ca^{2+} entry through voltage-dependent channels, transmitter release, and activation of the afferent fibers of the sinus nerve (Buckler and Vaughan-Jones, 1994; Ureña *et al.*, 1994). Monitoring cytosolic [Ca^{2+}] and dopamine release in intact single glomus cells demonstrates a characteristic relationship between PO_2 and transmitter secretion at the cellular level that is comparable to the relation described for the input-output variables in the carotid body. Thus, the properties of single glomus cells explain the sensory functions of the entire organ. We have recently demonstrated the existence of O_2-sensitive Ca^{2+} channels in glomus cells whose activity is also inhibited upon lowering PO_2 although in a strongly voltage-dependent manner (Montoro *et al.*, 1996). Both K^+ and Ca^{2+} channel activities are independently inhibited by hypoxia over a broad range of O_2 tensions (between 150 and 20 mmHg), however the properties and differential effects of O_2 on these ionic conductances help to explain why glomus cells secrete predominantly at low PO_2 values (below ≈ 70 mmHg). In the O_2-sensitive ion channels, hypoxia leads to specific biophysical alterations, which suggests that the changes of ion channel activity depend on O_2-sensing domains that regulate channel gating.

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References

Buckler, K. J. and Vaughan-Jones, R. D. (1994). *J. Physiol.*, 476, 423-428.

López-Barneo, J., Benot, A. R. and Ureña, J. (1993). *News Physiol. Sci.*, 8, 191-195.

Montoro, R., Ureña, J., Fernández-Chacón, R., Alvarez de Toledo, G. and López-Barneo, J. (1996). *J. Gen. Physiol.* 107, 133-143.

Ureña, J., Fernández-Chacón, R., Benot, A. R., Alvarez de Toledo, G. and López-Barneo, J. (1994). *Proc. Natl. Acad. Sc. USA*, 91, 10208-10211.

VENTILATORY CONTROL DURING SLEEP. A. I. Pack. 991 Maloney Building, Hospital of the University of Pennsylvania, Philadelphia PA, 19104-4283 USA. J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 17, 1997. S1 5

There are major changes in the ventilatory control system during sleep. These changes are different in non-REM and REM sleep. In non-REM sleep ventilation declines, a phenomenon that seems largely to be explained by the increase in upper airway resistance in this state. In stage 1-2 non-REM sleep, periodic breathing is also more likely to occur. This can be caused by chemical or state instability. The former is produced by high chemoreceptor gain, e.g., during hypoxia, and/or a long circulatory delay to the chemoreceptors. The latter, i.e., state instability, is more likely to occur when chemoreceptor gain is low and is the result of repetitive transient arousals from sleep. In REM sleep there are also marked changes in ventilatory output. Respiration is more irregular and there are episodes where there is a marked decline in respiratory motor output. Such episodes more commonly affect the motor output to upper airway muscles. Considerable insight into the mechanisms that produce these changes in REM sleep have come from study of the "carbachol model" of the atonia of REM sleep. In this model the atonia that occurs in REM sleep is induced by microinjection of the muscarinic agonist, carbachol, into the pontine tegmentum in decerebrate animals. This produces in lumbar motoneurons the same changes that occur in natural REM sleep, i.e., large state-specific IPSPs mediated by glycine. In contrast, although carbachol produces a marked decline in hypoglossal motor output, fast synaptic inhibition plays little, if any, role in this. This suggests that disfacilitation must play the major role. It is known that the firing of raphe cells that project to the hypoglossal motor nucleus declines both in REM sleep and following carbachol microinjection. Considerable evidence suggests that the decline in input from these cells plays a major role in the decreased motor output to airway dilator muscles during REM sleep. The evidence is as follows: the major neurotransmitter of raphe neurons, serotonin (5HT), is excitatory to hypoglossal motoneurons (mns); microinjection of antagonists of 5HT into the hypoglossal motor nucleus produces a decline in mn activity; levels of 5HT in the hypoglossal motor nucleus, as measured by microdialysis, are reduced following carbachol microinjection; microinjection of 5HT into the hypoglossal motor muscles markedly blunts the reduction in motoneuron activity produced by carbachol; TRH, another neurotransmitter in raphe neurons, is also excitatory to hypoglossal mns. Thus, one major component of the changes in ventilatory output that occurs in REM sleep is reduced activity of upper airway mns as a result of a decline in raphe neuronal firing in this state. Since the firing of these cells also declines in non-REM sleep, albeit to a lesser degree, this may also play a role in the changes in upper airway resistance that occur in this state. Other brainstem systems, such as locus coeruleus (LC), whose firing also declines in sleep, particularly REM sleep, are also likely to be important but the role of LC remains to be determined. (Original research supported by HL-42236).

NUTRITION AND AGING. J. Mataix and J. R. Huertas. Instituto de Nutrición y Tecnología de Alimentos. Universidad De Granada. 18071 Granada (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 19, 1997. S2 1

From a biological point of view, aging can be typified as a progressive incapacity to maintain internal medium in front of significant environmental changes. On the other hand, numerous theories have been set forth, related to cellular aging, that could be unified under the mitochondrial theory of aging.

The mitochondrial theory of aging gathers together the theory of scheduled degeneration based on the activation of the aging timer and the estocastic theory of disconnection between organs and structures due to the accumulation of multiple damages as age advances. The mentioned theory suggests that aging is the result of a cellular energetic degeneration due to causal somatic mutations in the mitochondrial DNA. This could rather be the result of the random distribution of these defects which show the heterogeneity of the phenomenon as well as of the deletions observed in the mitochondrial genoma than of the structural modifications caused during aging.

The oxidative damage appears to be among the possible causal agents of the mitochondrial damage mainly due to peroxidative reactions caused by free radicals that produce inactivation, mutation and even loss of mitochondrial DNA (Economos *et al.*, 1980; Miquel and Fleming, 1984). The major mutagenic reaction could be induced by malondialdehyde that binds amino acids groups of nucleic acids and with their bases, producing mutation, as well as by hydrogen peroxide that causes mutations and fragmentation of mitochondrial DNA (Samir *et al.*, 1972) and other agents (Graffi, 1940).

From the nutritional point of view, there is a possibility of acting of the dietary intake in the sense of substituting polyunsaturated fats for monounsaturated ones in view of delaying the effects of free radicals on the cellular aging mechanism.

This possibility is the result of researches recently conducted by our group. Specifically, in those situations typified by a high rate of free radical generation and therefore, of lipid peroxidation such as physical exercise, intake of xenobiotic foods and heated fat, virgin olive oil successfully protects mitochondrial and microsomal membranes from peroxidative modifications and mitochondrial DNA damages.

S2 2 MITOCHONDRIAL THEORY OF AGING: TIP OF THE ICEBERG OR TIP OF THE ICECUBE? S. DiMauro, E. Bonilla and E. A. Schon. Columbia-Presbyterian Medical Center. New York, NY (U.S.A.). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 20, 1997.

At the end of the 1980s, two lines of evidence converged to reinvigorate older suggestions that mitochondrial DNA (mtDNA) mutations may play an important role in human aging. First, histochemical studies of heart and muscle from normal aged individuals showed the presence of cytochrome c oxidase (COX)-negative fibers and biochemical studies of muscle suggested a gradual decline of respiratory chain activities with aging. Second, the discovery of multisystem human disorders due to rearrangements or point mutations in mtDNA called attention to the peculiarities of mitochondrial genetics and its possible relevance in aging. Soon thereafter, it was found that tissues from normal old subjects, and especially long-lived tissues with high energy requirements, such as brain, heart and muscle, contained low amounts (detectable only by PCR) of the same mtDNA deletions that are found in great abundance in patients with mitochondrial encephalomyopathies. While the percentage of each mutation was far too low (about 0.1 % of total mtDNA) to explain tissue dysfunction, the cumulative effects of multiple deletions and the possible contribution of point mutations could not be dismissed. In addition, neurologists got excited because a pathological "exaggeration" of this phenomenon (i.e. premature or excessive accumulation of mtDNA mutations) could explain many of the features of late-onset, non-mendelian, neurodegenerative diseases, such as Parkinson's disease (PD) or Alzheimer's disease (AD). This issue is riddled with controversies: in PD, there is good evidence that oxidative metabolism is impaired in the substantia nigra, but only flimsy evidence of abnormal accumulation of mtDNA deletions. Because the mitochondrial respiratory chain is the single greatest source of free radicals, and these may be overproduced by a defective respiratory chain, the "catastrophic mitochondrial theory of aging" envisions a vicious cycle whereby accumulating mtDNA deletions impair oxidative metabolism and increase production of oxygen radicals, which, in turn, further damage mtDNA. While the jury is still out on the direct responsibility of mtDNA mutations in aging, there is rapidly increasing evidence of oxidative energy decline and oxidative damage in the brain of patients with various neurodegenerative diseases, including PD, AD and amyotrophic lateral sclerosis. This opens interesting possibilities for investigation and therapeutic intervention.

MITOCHONDRIAL BIOENERGETICS IN AGING. G. Lenaz, C. Bovina, G. Parenti-Castelli, G. Formiggini, R. Fato, M. L. Genova, M. Merlo-Pich and F. Pallotti. Dipartimento di Biochimica "G. Moruzzi", Università di Bologna, 40126 Bologna (Italy). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 21, 1997. S2 3

The mitochondrial theory of aging proposes accumulation of somatic mutations in mitochondrial DNA (mtDNA) to be a key factor in determining the cellular energetic decline characterizing senescence. mtDNA encodes for 13 hydrophobic polypeptide chains of the four enzymatic complexes involved in energy transduction by H^+ translocation. Since 7 out of those chains are subunits of Complex I (NADH-Coenzyme Q (CoQ) reductase) it is predicted that this enzyme would be the most frequently affected. We have investigated the properties of Complex I in different systems.

(i) Mitochondrial populations (non-synaptic and synaptic) from brain cortex of 4- and 24-month-old rats. PCR analysis revealed that a 5 Kb deletion, analogous to the "common" deletion was present only in the old animals. Since the specific activity of NADH-CoQ reductase is underestimated using CoQ analogs as acceptors, we considered total aerobic NADH oxidation, related to NADH-CoQ reductase by the "pool equation". The largest changes were found in non-synaptic mitochondria, with a significant decrease of both Complex I content and turnover. Titration with the Complex I inhibitor, rotenone, exhibited a higher titer for half-inhibition (I_{50}) in the old rats. Since rotenone binds to hydrophobic subunits encoded by mtDNA, this finding is in accordance with the mitochondrial theory. The reason why only non-synaptic mitochondria are affected by aging may be related to their higher respiratory rate making them more prone to oxidative stress.

(ii) Mitochondrial membranes from platelets of young and old human individuals. NADH-CoQ reductase was not significantly different in the two groups, but there was a significant decrease of the rotenone sensitivity in the old individuals with a striking increase of the distribution of I_{50} to higher classes. The postulated energy decline was indirectly confirmed by the decreased inhibition of platelet aggregation in old individuals by the mitochondrial inhibitor antimycin in contrast with the effect of the glycolytic inhibitor deoxyglucose. Surprisingly, a mtDNA deletion different from the I "common" deletion was present in a consistent number of samples from old individuals.

S2 4 FREE RADICAL, ANTIOXIDANTS AND AGING. J. Miquel. Departamento de Farmacología y Terapéutica e Instituto de Neurociencias. Fac. Medicina. Alicante (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 22, 1997.

Many data suggest that aging and degenerative processes such as atherosclerosis and immunodeficiency derive from the oxidative stress caused by the free radicals which are a by-product of mitochondrial respiration. This concept is supported by our finding that aging of neurons and other differentiated cells is accompanied by progressive mitochondrial damage and accumulation of the age pigment lipofuscin from peroxidized mitochondrial debris. This organellar aging which is probably linked to mtDNA mutations, may play a key role in apoptosis and other types of cell death as well as on the bioenergetic and physiological decline that are most obvious signs of senescence.

The above justifies our present studies on dietary antioxidants and free radical scavengers which, according to preliminary data, increase the life span and stimulate the immune system of aging animals.

CNS CONTROL OF CARDIOVASCULAR FUNCTION: NEURAL MECHANISMS AND NOVEL MODULATORS. S3 1

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Cardiovascular reflexes act to maintain homeostasis. They are, however, amenable to modulation and the synaptic mechanisms underlying the changes in reflex efficacy have been a subject of major interest in my laboratory. In anaesthetized, paralysed and artificially ventilated cats we have investigated the role of the nucleus tractus solitarii (NTS) in mediating the arterial baroreceptor and chemoreceptor reflexes and the interactions between these two reflexes and those arising from laryngeal receptors (David Milner *et al.* 1995; Silva-Carvalho *et al.*; 1995a,b). Further we have determined the effects of stimulating the hypothalamic defence area on reflex function and NTS neurons that process inputs derived from these various reflex inputs. Whilst the neuronal interactions are often complex, clear patterns emerge that explain the interplay between reflexes and central drives. This presentation will seek to define both the synaptic mechanisms and their pharmacology, and to identify the physiological role of these interactions. Further reference will be made to novel neuro-modulators that have been shown to play a role in these synaptic processes.

These studies have been supported by grants from the Wellcome Trust and the British Heart Foundation.

Dawid-Milner, M. S., Silva-Carvalho, L., Goldsmith, G. E. and Spyer, K. M. Hypothalamic modulation of laryngeal reflexes in the anaesthetized cat: role of the nucleus tractus solitarii. *Journal of Physiology* (1995) 487.3, pp 739-749.

Silva-Carvalho, L., Dawid-Milner, M. S. and Spyer, K. M. The pattern of excitatory inputs to the nucleus tractus solitarii evoked on stimulation in the hypothalamic defence area in the cat. *Journal of Physiology* (1995a) 487.3, pp 727-737.

Silva-Carvalho, L., Dawid-Milner, M. S., Goldsmith, G. E. and Spyer, K. M. Hypothalamic modulation of the arterial chemoreceptor reflex in the anaesthetized cat: role of the nucleus tractus solitarii. *Journal of Physiology* (1995b) 487.3, pp 751-760.

S3 2 RECIPROCAL INTERACTIONS OF NPY AND ANG II RECEPTORS WITH α_2-ADRENOCEPTORS IN THE NUCLEUS TRACTUS SOLITARIUS. RELEVANCE FOR CENTRAL CARDIOVASCULAR REGULATION. S.-N. Yang, K. Fuxe, D. Fior, P. Hedlund, D. Ganten, J. A. Narváez and L. F. Agnati. Department of Neuroscience, Karolinska Institutet, 171 77 Stockholm (Sweden). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 24, 1997.

Neuropeptide Y (NPY), angiotensin II (Ang II) and adrenergic transmission lines in the nucleus tractus solitarii (NTS), the major relay nucleus involved in the cardiovascular reflex, play an important role in central cardiovascular control. The present work explored reciprocal interactions of NPY and Ang II receptor subtypes with α_2-adrenoceptors in the NTS of the normotensive Sprague-Dawley rat (SD), the normotensive Wistar Kyoto rat (WKY) and the spontaneously hypertensive rat (SHR) by using a cardiovascular analysis, quantitative receptor autoradiography, immunocytochemistry and *in situ* hybridization techniques.

α_{2A}-Adrenoceptors have been shown to be present in almost all catecholaminergic neurons in the brainstem including the NTS. NPY Y_1 or Ang II AT_1 receptor-like immunoreactivity (-LI) was found to be collocalized with tyrosine hydroxylase-LI in the NTS suggesting collocalization of these receptors with α_2-adrenoceptors in catecholaminergic neurons of the NTS.

In the NTS of the SD, the NPY Y_1 and Y_2 receptor agonists decreased the affinity and transduction of α_2-adrenoceptors. In contrast, α_2-adrenoceptor agonists increased NPY Y_2 receptor affinity but reduced the NPY Y_2 receptor functions, i.e., the NPY Y_2 receptor-mediated cardiovascular actions and the NPY-induced c-Fos expression in the NTS. NPY Y_2 receptor agonists reduced transduction over NPY Y_1 receptors in the NTS.

In the SHR, α_{2A}-adrenoceptor mRNA levels and α_2-adrenoceptor binding sites in the NTS were lower than those in the WKY. NPY in the NTS of the SHR had an increased potency to antagonize α_2-adrenoceptor function. This suggests that abnormal NPY/α_2-adrenergic receptor interaction may contribute to the development of hypertension.

In the NTS of the SD, activation of Ang II AT_1 receptors reduced the affinity and transduction of α_2-adrenoceptors, whereas α_2-adrenoceptor activation had the opposite effect on Ang II receptors.

Ang II differentially regulates α_2-adrenoceptors in the NTS of the WKY and the SHR. In the WKY, Ang II reduced the affinity and function of α_2-adrenoceptors. In the SHR, however, Ang II increased them. The opposite effect in the SHR may be one compensatory mechanism to counteract the development of high blood pressure in the SHR.

In conclusion, the present work provides evidence for reciprocal interactions of NPY and Ang II receptors with α_2-adrenoceptors in the NTS participating in cardiovascular regulation which are altered in relation to spontaneous hypertension. It introduces also the concept of hidden layer control through receptor circuits in central cardiovascular regulation.

THE ROLE OF GALANIN ON CENTRAL CARDIOVASCULAR CONTROL. S3 3

J. A. Narváez, Z. Díaz, P. B. Hedlund*, J. A. Aguirre, R. Coveñas, K. Fuxe* and S. González-Barón. Departamento de Fisiología, Facultad de Medicina Universidad de Málaga (Spain) and *Dept. of Neuroscience, Karolinska Institute, Stockholm (Sweden). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 25, 1997.

Galanin is a peptide originally isolated from the porcine intestine and widely distributed in the mammalian brain. Central galanin has been involved in multiple functions including neuroendocrine control, food intake, nociception, or higher brain functions. Recently, it has been demonstrated that N-terminal fragments of galanin are biologically active probably by acting on a different receptor subtype. The present investigate the possible involvement of galanin [gal-(1-29)] and the N-terminal galanin fragment (1-15) [gal(1-15)] on the central cardiovascular control (CVC) and their interactions with other substances involved in CVC as catecholamines, neuropeptide Y (NPY) or angiotensin II (AII).

Central administration of gal-(1-29) elicits a weak vasodepressor response and tachycardia, but the central administration of gal-(1-15) induces vasopressor and tachycardic responses. After coadministration of both substances gal-(1-15) reverses the vasodepressor effect of gal-(1-29) an even induces an increase in mean arterial pressure (MAP), but the tachycardic responses are not modified. Furthermore, gal-(1-15) decreases baroreceptor sensitivity whereas gal-(1-29) has no effect. Finally, the specific galanin receptor antagonist M40 abolishes the cardiovascular responses elicited by gal-(1-15) but no the cardiovascular effects induced by gal-(1-29).

Subthreshold doses of gal-(1-29) counteract the hypotensive and bradycardic effects induced by the α_2-agonist Clonidine (CLON) leading to an increase of MAP and heart rate (HK). *In vitro* studies show that CLON modifies the binding characteristics of [^{125}I]-gal-(1-29) and also gal-(1-29) modulates the binding of [^{3}H]-*p*-amino-Clonidine. However no interactions between gal-(1-15) and CLON have been observed. By other hand, coinjections of subthreshold doses of gal-(1-29) or gal-(1-15) with NPY induce a vasopressor actions with tachycardia, gal-(1-29) interacts mainly by NPY Y1 receptors and gal-(1-15) interacts almost exclusively with the NPY Y2 receptors. Interactions between galanin and AII have been also observed; the central cardiovascular responses to AII are enhanced by subthreshold doses of gal-(1-15), but gal-(1-29) have no effect. This interaction is mediated through the angiotensin AT1 receptor subtype.

Taken all data together it is demonstrated a role for galanin on CVC and it could be suggested that the cardiovascular actions may be mediated through two different galanin receptor subtypes. Furthermore, both galanin molecules modulates differentially the cardiovascular responses mediated α_2-adrenoceptors, NPY and AII, and these interactions seem to take place at the receptor level.

This work has been supported by CICYT (Sal91-0475) and DIGICYT (PB93-0992).

S3 4 ROLE OF DIFFERENT PONS REGIONS IN CARDIOVASCULAR CONTROL.

J. P. Lara, M. S. Dawid-Milner and S. González-Barón. Departamento de Fisiología, Facultad de Medicina, Málaga (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 26, 1997.

It is very well known that the pons is involved in the modulation of cardiovascular and respiratory systems. Two pontine structures, the dorsal parabrachial complex (PB) and the ventral A5 region (A5) participate in this central control. In the present study, both chemical and electrical stimuli have been delivered in these pontine areas in the anaesthetized rat to localize the sites from which modifications of cardiorespiratory activity are elicited, and to characterize the different modifications that were induced.

Rats were anaesthetized with pentobarbitone (60 mg kg^{-1}, i.p.) and, in most cases, artificially ventilated and paralysed (gallamine, 20 mg kg^{-1}, i.v.). Blood pressure, ECG and phrenic nerve activity were recorded. Multibarrel electrodes were positioned in both pons regions using stereotaxic coordenates. Cardiovascular and respiratory changes were analysed during electrical stimulation (10-40 μA, 0.4 ms pulses, 50 Hz, for 5 s) and glutamate injection (20-30 nl, 100 mM, pH 7.4 ± 0.1). Control saline injections were also made. In some animals, guanethidine (10 mg kg^{-1}, i.v.) and phenylephrine (15 μg kg^{-1}, i.v.) were given. Electrical lesions or pontamine sky blue injections marked the site of stimulation.

PB stimulation evoked a common pattern of increase in blood pressures with a small yet significant increase in heart rate with electrical stimulation ($p < 0.001$, both cases; 20 cases; paired sample test) and glutamate injections ($p < 0.001$ and $p < 0.01$, respectively; 16 out of 20 cases). This response involved an increase of sympathetic activity, since it was completely abolished by guanethidine. The coexistence of increases in both blood pressure and heart rate indicates the presence of inhibition of the heart rate component of the baroreflex and/or increase in cardiac sympathetic drive; in fact, PB stimulation overrided the baroreceptor-induced bradycardia (phenylephrine). A5 stimulation also induced cardiovascular modifications. At all sites, electrical stimulation evoked a response of increase in blood pressure and heart rate ($p < 0.001$ and $p < 0.05$, respectively). The response to glutamate injection was variable. In most cases, identical to the effects of electrical stimulation at the same site: increases in blood pressure and heart rate ($p < 0.01$ and $p < 0.05$, respectively; 21 out of 29 cases); in some animals, the response was biphasic with a transient pressor response followed by a small depressor response (8 out of 29 cases); this depressor component was not elicited by baroreflex activation as an increase in heart rate ($p < 0.05$) occurs simultaneously during both the pressor and depressor response. The diversity of cardiovascular responses may reflect the diversity of neurons located within this region. Guanethidine abolished all this cardiovascular modifications. The reflex bradycardia induced by phenylephrine was reversed by A5 stimulation, suggesting a modulation of the baroreceptor reflex.

These results show some cardiovascular modifications that can be evoked from the PB and A5 region. The similarities of the changes elicited from both areas may suggest a functional relationship between these pontine regions. They also imply an action at the level of the ventrolateral medulla that can be further investigated.

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BRAIN ANGIOTENSINS IN THE PATHOGENESIS OF ARTERIAL HYPERTENSION. S3 5
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The successful insertion of the Ren-2 gene into the genome of Sprague Dawley rats has allowed a successful test of a hypothesis regarding the role of the brain renin angiotensin system in the evolution of experimental arterial hypertension. Immunoreactive renin was visualized in neurons of the paraventricular, supraoptic and arcuate nuclei of the hypothalamus, and in the choroid plexus and subfornical organ within the III ventricle of transgenic positive [Tg(+)] adult hypertensive rats. In contrast, renin messenger RNA was discretely localized to cells at the point where the choroid plexus and subfornical organ attached to the ependymal lining of the cerebral ventricles during probing with a ^{35}S-labeled 226 nucleotide riboprobe complementary to the Ren-2 rnRNA. The detection of renin in the brain of Tg(+) hypertensive rats was associated with the presence of high tissue concentrations of Ang II and Ang-(1-7) in both the hypothalamus and dorsal medial medulla oblongata. The functional importance of increased brain levels of Ang II to the maintenance of hypertension was verified by the demonstration that injection of a specific Ang II monoclonal into the cerebral ventricles of Tg(+) rats elicited rapid and dose-dependent decreases in arterial pressure. Cerebroventricular administration of a specific Ang-(1-7) antibody elicited instead dose-dependent rises in arterial pressure. The combination of anatomical, biochemical and physiological approaches to the investigation of the role of the brain angiotensins has revealed a central role of this tissue system in the production of a genetic form of high blood pressure.

S3 6 MEDULLARY AND CEREBELLAR CARDIOVASCULAR CENTERS AND NEUROGENIC NEUROPROTECTION. D. J. Reis. Cornell University Medical College, New York, NY (USA). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 28, 1997.

Centers of brainstem and cerebellum involved in regulating arterial pressure also act to protect the brain from ischemia and/or hypoxia. Two principal mechanisms by which intrinsic neuronal networks can offer protection have been uncovered. The first, hypoxemic cerebral vasodilatation, is mediated by activation of oxygen sensitive neurons in the rostral portion of the rostral lateral medulla (RVLr). When excited, RVLr neurons increase cortical cerebral flow (rCBF) without increasing cerebral metabolism. The effect is mediated by activation of neurons in the caudal RVL (RVLc) over an intrinsic neuronal pathway engaging neurons in deep forebrain nuclei. The second, central neurogenic neuroprotection, is elicited by stimulating the cerebellar fastigial nucleus (FN) for 1 h. Such stimulation site-specifically reduces, by over 50 % the magnitude of infarction produced by focal or global cerebral ischemia even when lesions are produced 10 days after stimulus trial. Neuronal salvage is not related to changes in rCBF or metabolism, but appears to relate to (1) increased neuronal excitability mediated by opening K^+ channels, (2) a marked reduction of cerebral microvessels to express proinflammatory molecules and (3) induction of several anti-inflammatory elements in the brain. The brain contains networks whose activation can protect the organ from brief and or prolonged hypoxic/ischemic injury. The central pathways may relate to mechanism governing the integrate autonomic and behavioral reflexes linked to oxygen-conserving cardiovascular reflexes of diving mammals.

TEMPERATURE CONTROL AND BRAIN NEUROTRANSMITTERS. F. Mora. S4 1
Dept. Physiology. Fac. Medicine. Complutense University. Madrid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 29, 1997.

In 1963 Feldberg and Myers proposed that Noradrenaline (NA) and Serotonine (5-HT) released into the hypothalamus mediated heat loss and heat gain mechanisms respectively. Since then, these monoamines, as well as dopamine, acetylcholine, GABA and other amino acids have been suggested to be involved in the control of body temperature within the anterior-preoptic hypothalamus (PO/AH). Also the simultaneous release of several neurotransmitters i.e. DA, NE and Aspartic acid (ASP) has been shown to be released into the PO/AH during heat exposure. More recent data has also suggested the involvement of glutamic acid (GLU) not only in the control of temperature regulation but in the deleterious effects produced by heat stroke in the PO/AH and other areas of the brain. A new perspective in brain research has been the functional interaction between different types of neurotransmitters in well defined areas of the brain. In the neostriatum as well as in the PO/AH the interaction between GLU and DA has been suggested. Also the interaction between excitatory neurotransmitters (GLU, ASP) and inhibitory neurotransmitters (GABA, Taurine (TAU)) in these last two areas of the brain has been proposed. In particular and in the PO/AH, the interaction between GLU, ASP, GABA and TAU has recently been proposed to be part of the neurochemical substrates underlying temperature regulation. These recent data provides a new perspective for the analysis of the neurochemical substrates of temperature regulation in the brain.

1) Del Arco, A., Segovia, S., Mora, F. Endogenous glutamate produces an increase in extracellular concentrations of dopamine, GABA and Taurine in striatum of the freely moving rat: correlational studies. In: Monitoring molecules in Neuroscience. González-Mora, J. L., Borges, R., Mas, M. (eds.) University of La Laguna. 253-254 (1996).
2) Expósito, I., Mora, F., Oaknin S. Dopamine-glutamic acid interaction in the anterior hypothalamus: modulatory effect of melatonin. *Neuro Report*, 6, 661-665 (1995).
3) Expósito, I., Mora, F., Zisapel, N., Oaknin, S. The modulatory effect of melatonin on the dopamine-glutamic acid interaction in the anterior hypothalamus during aging. *Neuro Report* 6, 2399-2403 (1995).
4) Mora, F., Porras, A. Interactions of dopamine, excitatory amino acids and inhibitory amino acids in the basal ganglia of the conscious rat. In: The Basal Ganglia. IV: New Ideas and data on structure and function. Percheron, G., McKenzie, J. S. and Feger, J. (eds.) *Advances in Behavioral Biology*, 41, 441-447 (1994).
5) Porras, A., Mora, F., Dopamine-glutamate-GABA interactions and aging: studies in striatum of the conscious rat. *Eur. J. Neurosci.* 7, 2183-2188 (1995).

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S4 2 EFFECTS OF HYPERTHERMIA ON ROS AND ENDOTOXEMIA IN THE RAT.

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Previous research has provided evidence that whole body hyperthermia produces regionalized hypoxia in splanchnic tissues and vasodilation of the superior mesenteric artery. The purpose of this study was to evaluate the effects of whole body hyperthermia on the concentration of reactive oxygen species (ROS) and endotoxin in portal venous blood. Male Sprague Dawley rats were fitted with portal vein catheters and allowed to recover for 48 hours. The unanesthetized animal was subsequently exposed to an ambient temperature of 40 °C. Portal vein blood was sampled as colonic temperature (T_c) rose from 37 °C to 43.5 °C and analyzed for ROS and endotoxin using electron paramagnetic resonance (EPR) and a chromogenic amoebocyte lysate assay, respectively. EPR analysis revealed increased concentrations of ceruloplasmin (acute phase antioxidant protein), semiquinone radical (biomarker of oxidatively stressed cells), and hemoglobin-nitric oxide. Portal venous endotoxin concentration rose from a control value of 28±7 pg/ml to 62±4 pg/ml at T_c 41.5 °C. These data provide evidence that hyperthermia generates reactive oxygen species that contribute to impaired intestinal barrier function leading to portal endotoxemia. They further suggest that the release of nitric oxide in the hyperthermic animal may contribute to splanchnic vasodilation.

ROLE OF HYPOTHALAMIC NEURONAL NETWORKS IN THERMOREGULATION. S4 3

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The regulation of body temperature is partially controlled by the preoptic-anterior hypothalamus (PO/AH), an area containing warm sensitive, cold sensitive and temperature insensitive neurons. Intracellular recordings have been conducted to understand the cellular properties and synaptic connections responsible for different types of neuronal thermosensitivity. In the PO/AH, neuronal cold-sensitivity often can be attributed to synaptic inputs from nearby thermosensitive neurons. In contrast, neuronal warm-sensitivity is intrinsic to many neurons and is determined by a depolarizing prepotential that precedes each action potential. This prepotential is present in both warm sensitive and temperature insensitive neurons, but temperature has different effects on the prepotential in these two types of neurons. In warm sensitive neurons, the rate of rise of the prepotential is increased by warming, and this produces an increased firing rate. On the other hand, warming has little or no effect on the prepotential of temperature insensitive neurons. Warm sensitive neurons also receive inhibitory and excitatory synaptic input from temperature insensitive neurons, and the predominant postsynaptic potentials are IPSPs. This inhibitory input may enhance the warm-sensitivity of some neurons, since cooling increases input resistance and IPSP amplitudes. Therefore, even though IPSP frequencies do not change, cooling can decrease firing rates by increasing IPSP amplitudes. This change in firing rate is not sufficient to solely account for warm-sensitivity; however, it can contribute to the thermosensitivity of a neuron that is intrinsically warm sensitive.

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S4 4 ROLE OF CYTOKINES IN THE NEUROMODULATION OF FEVER. F. J. Miñano. Departamento de Farmacología, Pediatría y Radiología, Facultad de Medicina, Universidad de Sevilla, 41009 Sevilla (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 32, 1997.

Exposure to infectious or inflammatory agents is followed by the appearance of circulating polypeptide mediators such as cytokines, which contribute to adaptive changes for the defense of the host. It has been proposed that endogenous pyrogens such as interleukin-1β (IL-1β) and IL-6, synthesized from monocytes and macrophages and released into the circulation, might penetrate the fenestrated endothelia of capillaries in the organum vasculosum laminae terminalis (OVLT), bind to their receptors located on astrocytes that tightly surround the vascular network, and trigger the synthesis and release of prostaglandins (PGs), particularly PGE_2. This PGE_2 reaches and activates predominantly EP-1 receptors located on thermosensitive neurons in the adjacent anterior hypothalamic, preoptic area (AH/POA) causing the autonomic and behavioral responses characteristic of fever. However, where PGs are involved in the pathway is unclear. Thus, the fever produced by a pyrogenic cytokine such as macrophage inflammatory protein-1β (MIP-1β) is unaffected by inhibitors of cyclooxygenase in the rat, whereas the fever evoked by IL-1 or IL-6 is antagonized or attenuated by these inhibitors. It has been demonstrated that MIP-1β is produced in the OVLT and the AH/POA in response to bacterial lipopolysaccharide (LPS) and that is essential for the integral development of a febrile response following a bacterial pyrogen. More recently it has been demonstrated that IL-1β and IL-6 are required for both PGE_2 and LPS induced fever. However, MIP-1β is not required for the hyperthermic response to centrally injected PGE_2. Thus, although the complexity of this system makes difficult the interpretation of the role of these cytokines in PGE_2-induced hyperthermia, the complex pattern of cross-induction between these cytokines and PGE_2, which control and regulate the release of each other may be example of natural feedback mechanism for the co-ordinated development of a fever dependent of the cyclooxygenase pathway involving both synergistic and inhibitory processes within hypothalamic thermoregulatory neurons. The divergent nature of the pyrogenicity of MIP-1β and other cytokines would provide an additional level for the functional control of the febrile response. Thus, several distinct pathways for the induction of fever might exist within the brain. Consequently, although a model of fever, involving at least two different pathways in the rat, has been proposed: 1) LPS may induce the release of cytokines (IL-1β, IL-6) and PGs, which control and regulate the release of each other; and 2) a second pathway, independent of PGs, in which fever is directly related to the hypothalamic presence of MIP-1β, the possibility of an alternate or different pathway in which a role for PGE_2 as potentiator of pyrogenic cytokines switching the production of selected cytokines, such as IL-6 and/or IL-1β, into the hypothalamic cells, rather than an obligatory final step in the production of fever, should be taken into account.

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ROLE OF NO AND PG's IN VOLUME- AND PRESSURE-INDUCED NATRIURESIS. S5 1

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J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 33, 1997.

Studies were undertaken with the purpose of defining the role of NO and PG in mediating the natriuresis produced by both volume expansion and increase in pressure.

The IV infusion of a large amount of NaCl (10 % body weight) in anesthetized dogs evoked an increase in mean arterial pressure by 13±2 mmHg and a total elevation of urinary sodium excretion (UVNA) of 524±86 pmol/min. These yield a conductance of 40 mEq of sodium excreted/each mmHg of increase in blood pressure. After the blockade of prostaglandin of PG synthesis, identical volume expansion produced a natriuresis of 219 mEq/mmHg. Such a significant increase in sodium excretion conductance was most probably due to a concomitant fall in the circulating levels of plasma renin activity (from 3±0.2 ng/ml/min to 0.8 ng/kg/ml) and endothelin (from 12±1 pg/ml to 9±1 pg/ml).

In contrast the blockade of NO synthesis was followed by a significant elevation of endothelin from 11±1 to 16±1 pg/ml and plasmin renin activity (from 6±1 to 9±1 ng/ml) all of which decrease the conductance of volume expansion to 12 mEq of sodium per mmHg. After the simultaneous blockade of NO and prostaglandins, volume expansion was followed by 15±6 mmHg of increase in blood pressure, natriuresis was blunted by 70 %. The results show that NO plays a critical role in promoting sodium excretion during volume expansion without altering blood pressure. In the absence of NO, salt loading produced a significant increase in MAP that enhances sodium excretion (pressure natriuresis) by stimulating PG synthesis.

It has been shown by other investigators that changes in medullary blood flow could be responsible for regulating sodium excretion. However, we found that blockade of prostaglandin synthesis that under normal conditions fail to alter sodium excretion produced a marked 75 % decrease of medullary blood flow. In contrast, administration of nitric oxide synthesis which affects sodium excretion produced only a 30-35 % decrease in medullary blood flow.

In addition, studies of intrarenal distribution of blood flow and alterations in tubular dynamics were estimated with a use of 3-dimensional computerized tomography. We found that changes in renal perfusion pressure within the range of autoregulation failed to alter intrarenal distribution within the renal cortex. However, it produced marked changes in the papillary flow. Interestingly, in the inner medullary zone outlying the papillary area, blood flow experienced changes of opposite direction then those seen in the papilla. These changes were associated with marked decrements in tubular sodium reabsorption in both proximal, thick ascending loop of Henle and distal tubules.

These results shows that very small changes in medullary flow are associated with significant changes in sodium tubular reabsorption.

It was also noticed that the administration of L-name renders the maintenance of renal circulation solely dependent on prostaglandins because the subsequent blockade of prostaglandin was followed by a marked fall in GFR and sodium excretion approaching renal failure. In contrary, such an effect is not observed when this suppression of NO synthesis was performed after removing the synthesis of prostaglandin.

S5 2 CONTRIBUTION OF INTRARENAL NITRIC OXIDE TO THE MECHANISM OF PRESSURE NATRIURESIS. L. G. Navar and D. S. A. Majid. Department of Physiology, SL39, Tulane University School of Medicine, New Orleans, LA 70112 (USA). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 34, 1997.

Recent studies have indicated that intrarenal production of nitric oxide (NO) exerts an important role in the regulation of renal hemodynamics and excretory function. Studies in dogs have demonstrated that inhibition of NO synthase by intra-arterial administration of nitro-L-arginine (NLA) reduces renal blood flow (RBF) without altering GFR or autoregulatory efficiency of RBF, and reduces sodium excretion without reductions in filtered load. In addition, NO synthase inhibition markedly suppresses the slope of the relationship between renal arterial pressure and sodium excretion (pressure natriuresis). Further studies have shown that a constant intra-arterial infusion of a NO donor compound, S-nitroso-n-acetylpenicillamin (SNAP) in dogs treated with NLA produces diuretic and natriuretic responses but fails to restore the slope of the relationship between arterial pressure and sodium excretion. These data indicate that active alterations in intrarenal NO activity, rather than the constant permissive presence of NO, during changes in renal arterial pressure are required for full expression of the arterial pressure induced changes in sodium excretion. It has further been demonstrated that there is a direct relationship between changes in arterial pressure and urinary excretion rate of nitrate and nitrite, which is used as a marker for endogenous NO activity. More recent experiments using an NO-sensing electrode inserted into the renal cortex have provided further data supporting a direct relationship between arterial pressure and the renal tissue concentration of NO. The NO-sensing electrode is responsive to NO agonists such as acetylcholine, bradykinin, and ATP, and to NO donors such as SNAP. Alterations in renal arterial pressure within the autoregulatory range led to parallel changes in intrarenal NO activity which were correlated with the changes in sodium excretion as well as urinary excretion rate of NO metabolites, nitrates and nitrites. Extensive experiments using single fiber laser-Doppler needle flow probes in the different regions in the kidney have documented that the effects of arterial pressure on sodium excretion occur in the presence of highly efficient autoregulation of cortical, outer medullary and inner medullary blood flow. Collectively, these data are most consistent with the hypothesis that acute changes in renal arterial pressure elicit parallel changes in intrarenal NO production which may directly alter tubular sodium reabsorption rate to manifest the phenomenon of pressure natriuresis.

ROLE OF NITRIC OXIDE AND PROSTAGLANDINS IN THE REGULATION OF THE RENAL HEMODYNAMIC AND EXCRETORY FUNCTION. S5 3

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The role of nitric oxide (NO) in the regulation of the renal hemodynamic and excretory function has been suggested by the results obtained in many studies. It has been proposed that there is a tonic release of NO that plays an important role in mediating the renal response to changes in arterial pressure; to the administration of renal vasodilators and to the intake of a high protein meat meal. There are also some evidences showing that the reduction of NO synthesis induces the development of a sodium sensitive hypertension. Results recently reported by our group also suggest that there is an important interaction between NO and other intrarenal hormones in the acute and long-term regulation of renal function.

In studies performed in anesthetized dogs, it was observed that the administration of a low dose of a NO synthesis inhibitor or a prostaglandins (PG) synthesis inhibitor does not induce changes in arterial pressure and renal function. However, the simultaneous acute inhibition of NO and PG synthesis induced an increase of arterial pressure and a 40 % decrease in renal blood flow and glomerular filtration rate that seem to be secondary to the vasoconstriction induced by the endogenous levels of Angiotensin II (Ang II). The acute inhibition of NO and PG synthesis also induced a significant increase of sodium reabsorption that was independent of the intrarenal Ang II levels. The interaction between NO and PG found in the acute studies previously mentioned, has also been suggested to occur in the long-term regulation of renal function. In long-term experiments, it was found that the simultaneous inhibition of NO and PG synthesis during several consecutive days induced a renal vasoconstriction and an increase in sodium reabsorption that was significantly greater than that induced by the long-term reduction of NO or PG synthesis. The regulation of renin release seems to be also altered during the long-term reduction of NO and PG synthesis.

An important role of NO in the regulation of the renal response to increments in the intrarenal Ang II levels has been suggested by the results obtained by our group. It was found that the vasoconstrictor and antinatriuretic effects induced by Ang II are highly potentiated when the renal production of NO is slightly reduced. The antinatriuretic effects secondary to the reduction of NO synthesis seems to be secondary to an increment of sodium reabsorption in the proximal tubule and to a decrease in medullary blood flow.

The results suggesting that endogenous NO modulates the effects induced by Ang II or secondary to the administration of cyclooxygenase inhibitors may have important clinical implications because it is known that there are situations like aging where NO synthesis seems to be reduced and the intake of antiinflammatory drugs is elevated.

S5 4 EFFECTS OF ENDOTHELIN ON GLOMERULAR FUNCTION AND MESANGIAL CELL ACTIVATION. J. M. López-Novoa. Instituto Reina Sofía de Investigación Nefrológica, Depto. de Fisiología y Farmacología, Universidad de Salamanca, Salamanca (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 36, 1997.

Endothelin (ET) is a 21-residue peptide that has been isolated from the supernatant of cultured vascular endothelial cells and shown to be one of the most potent vasoconstrictors ever known. Three genes related to ET are present in the human, porcine and rat. One of these three genes encodes the original porcine/human ET (hereafter called ET-I). The other two genes encodes ET-2 and ET-3, which are different in two and six amino acid residues, respectively, from ET-1. ET-1 was originally considered to be a novel humoral regulator of the cardiovascular system. Moreover, accumulating evidence indicates that ET actually induces diverse biological responses in these tissues. One of these actions other than cardiovascular control is a growth-promoting activity.

There exist two distinct classes of ET binding sites, ET_A-R and ET_B-R respectively, the first one with a higher affinity for ET-1 and ET-2 than for ET-3 and the second one with a higher affinity for ET-3 than for ET-1 or ET-2. ET_A-R activation (mainly by ET-1) induces cell contraction and cell proliferation, whereas ET_B-R activation (mainly by ET-3) induces vasodilatation an growth modulation.

The vasoconstrictor action of ET-1 includes all arterial beds, but renal circulation is especially susceptible to vasoconstrictor effects of exogenous ET-1, probably due to a great predominance of ET_A-R receptors. ET-1 reduces renal blood flow due to a marked increase in renal vascular resistance. The effect of ET-1 is similar in the afferent and the efferent arteriole, thus without significant changes in intraglomerular capillary pressure. However, ET-1 administration reduces glomerular filtration rate by inducing a decrease in ultrafiltration coefficient (Kf). This decrease in Kf is produced by a marked contraction of mesangial cells, which has been also demonstrated *in vitro*. Increased local ET-1 production during renal ischemia or nephrotoxicity are responsible for marked renal vasoconstriction and Kf decrease in these circumstances. In addition, ET-1 is also able of inducing smooth muscle and mesangial cell proliferation and activation. Mesangial cell activation includes the modification of its phenotypical status, inducing the synthesis of several inflammatory mediators as platelet activating factor, reactive oxygen radicals and pro-inflammatory cytokines and growth factors. The consequence of mesangial cell activation is the development of inflammatory processes into the glomeruli, leukocyte infiltration and an increase in extracellular matrix synthesis. These effects of endothelin can play a major role in the structural changes observed within the glomeruli in several chronic pathologic immune or non-immune mediated processes, such as glomerulonephritis, hypertension- or diabetes-associated glomerulosclerosis or chronic interstitial nephritis.

ENDOTHELIN, THE KIDNEY, AND HYPERTENSION. J. P. Granger, Department of Physiology and Biophysics, Univ. of Mississippi Medical Center, Jackson, MS 39216-4505 USA). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 37, 1997. S5 5

The endothelium is thought to play an important role in the regulation of arterial pressure through the release of a variety of relaxing and contracting factors. These factors have also been suggested to be involved in various aspects of hypertension. One of the contracting factors, endothelin-l, is the most potent endogenous vasoconstrictor ever identified. Endothelin is synthesized in endothelial, smooth muscle, cardiac, and renal tubule cells in response to humoral and mechanical stimuli. Although most of the endothelin synthesized in the endothelium is released towards the underlying smooth muscle cells, some endothelin is secreted into the blood stream and affects distant organs. Despite the evidence that plasma levels of endothelin-1 are only slightly elevated in experimental and human hypertension, long-term elevation of plasma endothelin within the pathophysiological range causes significant reductions in renal pressure natriuresis and hypertension. Chronic increases in plasma endothelin within the renal circulation also produces significant hypertension. Increased ET synthesis within the blood vessels without changes in plasma ET has been reported to occur in several animal models of high blood pressure, especially in the malignant forms of hypertension. Long-term administration of endothelin receptor antagonists reduce or attenuate the hypertension in many of these animal models. In addition to playing a potential role in the development and/or maintenance of hypertension, increasing experimental evidence indicates that ET may participate in the vascular and cardiac hypertrophy associated with hypertension. Thus, growing evidence from animal experimentation indicates that ET may be involved in various aspects of the hypertension process. The use of specific ET receptor antagonist and/or synthesis inhibitors should allow a better understanding of the importance of ET in human hypertension.

CORTISTATIN, A NOVEL SOMATOSTATIN-RELATED NEUROPEPTIDE WITH DISTINCT PHYSIOLOGICAL PROPERTIES. S6 1

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We report the cDNA cloning of an mRNA encoding the precursor of a novel neuropeptide, cortistatin, whose deduced amino acid sequence shares a strong structural similarity with somatostatin. Rat preprocortistatin is synthesized as a 112 amino acid precursor, containing a 21-residue secretory signal peptide and two potential cleavage sites that would give rise to cortistatin-29 and cortistatin-14. Cortistatin 14 shares 11 of 14 amino acids with somatostatin-14, including the FWKT residues, critical for somatostatin binding to its receptors and two cysteines that are likely to render the peptides cyclic. The C-terminus of preprocortistatin is fully conserved between rat and mouse, and the mouse gene maps to chromosome 4 between *wld* (wallerian degeneration) and *swe* (slow wave epilepsy).

Preprocortistatin mRNA is expressed postnatally in the rat brain in a distinct population of cortical and hippocampal GABAergic interneurons that partially overlap with those expressing somatostatin. Synthetic cortistatin binds to somatostatin receptor subtypes *in vitro* with different selective affinities. Like somatostatin, superfusion of cortistatin-14 induces neuronal hyperpolarization and enhances the voltage-dependent potassium M-current in the hippocampal slice preparation as seen by voltage- and current- clamp recordings.

Intracerobroventricular administration of cortistatin decreases locomotor activity and enhances slow-wave sleep. Preprocortistatin mRNA steady state levels are increased 3-fold in sleep-deprived rats, as compared to controls, suggesting a physiological role for cortistatin in the regulation of cortical activity and sleep induction. Because acetylcholine is one of the key neurotransmitters that regulate the different phases of sleep, we investigated the interaction between cortistatin and the cholinergic system. In contrast to somatostatin, cortistatin antagonizes the effects of acetylcholine on different measures of cortical excitability, suggesting a mechanism of cortical synchronization related to cognition and sleep.

S6 2 THYROID HORMONES AND BRAIN GENE EXPRESSION. J. Bernal, B. Morte, M. A. Iñiguez, T. Iglesias, A. Guadaño-Ferraz, P. Vargiu, L. F. García-Fernández, P. Lorenzo and A. Muñoz. Instituto Investigaciones Biomédicas, Arturo Duperier 4, 28029 Madrid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 40, 1997.

Thyroid hormones (TH) are essential for proper brain development in mammals. In the human being the lack of TH during the perinatal period, as a consequence of Congenital Hypothyroidism or of a severe deficiency of iodine intake, leads to cretinism, a syndrome of mental deficiency which may be associated to severe neurological deficits. The action of TH is mediated by the binding of triiodothyronine (T3) to nuclear receptors (T3Rs) and the modulation of gene expression. The receptors are members of a large family of ligand-modulated transcription factors which include the receptors for steroids, retinoids, vitamin D3, and other molecules without known ligands (orphan receptors). Nuclear receptors bind to response elements (RE) in the regulatory regions of their target genes as homodimers or heterodimers with RXR, the receptor for 9-cis retinoic acid.

Despite the profound effects of thyroid hormone on brain maturation, until recently no brain target genes of TH were known. In our laboratory we used molecular biological techniques to identify such targets. We used differential screening and subtractive hybridization techniques, as well as a modification of a genomic PCR approach to select genes expressed differentially in the brains of normal and hypothyroid rats. The TH-regulated genes found, which are probably a small fraction of the TH-dependent genes *in vivo*, include those encoding the major proteins of myelin (MBP, PLP, MAG), as well as neuronal genes, such as the PKC substrate RC3/neurogranin, PGD2 synthase, NCAM, NGF and other unidentified genes. Regulation of expression of these genes by TH is complex: some genes, as RC3, displaying cell-specific regulation, whereas others (myelin) require T3 for expression only during the first postnatal weeks in the rat. This suggests that unknown factors act in concert with the T3R to modulate the expression of these genes. Regulation in most cases is at the level of transcription, which may be demonstrated by run-on assays *in vivo* or in cultured cells. In addition, specific response elements were found in some genes (for example, NCAM), whereas in others the T3RE has not yet been identified (RC3).

It is concluded that, as in other tissues, thyroid hormone affects brain maturation by regulating gene expression during critical periods of postnatal development in the rat. The regulated genes cannot be grouped into a single category, which explains why the effects of TH on the brain are so diverse.

EFFECTS OF SEX STEROIDS ON BRAIN CELLS: INTERACTIONS WITH INSULIN-LIKE GROWTH FACTOR-I. S6 3

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Sex steroids have developmental trophic actions on neurons and glial cells and activational effects in the adult brain. Part of these effects are due to the direct activation by the hormones of their own nuclear receptors. Sex steroids may also have rapid non-genomic effects on neuronal membranes. Furthermore, it has been proposed that sex steroids may interact with peptide trophic factors to induce part of their biological effects. We are studying the interaction of sex steroids with insulin-like growth factor-I (IGF-I), a cytokine that is locally synthesized by glia and neurons of the hypothalamus, as well as other brain areas and has prominent trophic actions, stimulating survival, proliferation and differentiation of specific neural cell populations. IGF-I may also participate in neuroendocrine events at the level of the hypothalamus since it has been shown to be involved in the feed-back regulation of growth hormone by affecting the synthesis or the release of growth hormone releasing hormone and somatostatin by hypothalamic neurons. IGF-I may also affect the reproductive axis by modulating the release of gonadotropin hormone releasing hormone by hypothalamic cells and, therefore, the release of gonadotropins. Cellular mechanisms mediating sex steroid actions on brain cells have been studied extensively in the rat arcuate nucleus (AN), a hypothalamic center involved in the feed-back regulation of growth hormone and gonadotropins. During the preovulatory and ovulatory phases of the estrous cycle there is a transient disconnection of inhibitory synaptic inputs to the somas of AN neurons. This synaptic remodeling is induced by estradiol, blocked by progesterone, and begins with the onset of puberty in females. Astroglia appear to play a significant role in the hormonal effects on neuronal connectivity by regulating the amount of neuronal membrane available for the formation of synaptic contacts and by releasing soluble factors, such as IGF-I. IGF-I levels in glial cells of the rat AN are sexually dimorphic, increase with the onset of puberty and are regulated by perinatal levels of testosterone and adult levels of estradiol and progesterone. Changes in IGF-I levels in the AN are linked to the reorganization of the glial cytoskeleton and to the modulation of synaptic connectivity by glial cells and appear to be mediated by hormonal modifications of the uptake of IGF-I by hypothalamic glia from blood or cerebrospinal fluid. *In vitro*, both estradiol and IGF-I increase the survival and differentiation of developing fetal rat hypothalamic neurons. The effect of IGF-I can be blocked by estrogen receptor (ER) antagonists and by the inhibition of ER synthesis by antisense oligonucleotides. In turn, the effect of estradiol is blocked by the inhibition of IGF-I synthesis.

These findings indicate that sex steroids modulate IGF-I levels in the neuroendocrine hypothalamus and that estrogen-induced activation of the ER in developing hypothalamic cells requires the presence of IGF-I. Furthermore, the results suggest that both estradiol and IGF-I use the ER as a mediator of their trophic effects on hypothalamic neurons. Therefore, sex steroids and peptide trophic factors appear to interact to exert their effects on brain cells.

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S6 4 NEURAL REGULATION OF GROWTH HORMONE SECRETION. J. A. F. Tresguerres and J. Devesa. Department of Physiology, Medical School, University Complutense, Madrid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 42, 1997.

Growth hormone GH has a pulsatile secretion pattern, as the result of the interaction of stimulatory GHRH and inhibitory somatostatin (SS) hypothalamic peptides. Both hormones arrive at the pituitary gland via the hypophyseal portal system, and are under the control of suprahypothalamic structures and also depend on plasma GH levels. Both, metabolites (blood glucose, FFA) and neurotransmitters (cholinergic) have been shown to markedly influence GHRH and especially SS regulation. All these effects seem to be mediated at the end by the central adrenergic system in which $\alpha 2$ inhibits, and β stimulates SS neurons. The stimulatory role of $\alpha 2$ adrenergic agonists, as clonidin, are thus due to the inhibition of SS instead of the stimulation of GHRH as was previously considered. Peripheral hormones play also a permissive role in the regulatory process of GH, being the most important thyroid, sexual and glucocorticoid hormones. Thyroid hormones play a role in the pituitary GH synthesis whereas sexual hormones seem to be responsible for the different secretion pattern in males and females. The role of glucocorticoids remain controversial, since they have been shown to increase transcription and expresion of GH gene, and on the other hand inhibit GH secretion by stimulating SS under β adrenergic control. GHRH has been found not only to stimulate GH synthesis and secretion but also to play an important role in pituitary somatotrophic cell differentiation. This effect is mediated by pit-l. Normal rats and mice show a submaxillar cell transdifferentiation with GH and prolactin secretion when submitted to high local GHRH concentrations. Pit-l deficient dwarf mice are not showing this transformation. The long term regulation of GH has some interesting points that need also to be investigated, since a certain "down regulation" at pituitary level has been found when GHRH stimulation is maintained for long time. A new role for SS can be postulated.

This work has been possible through grants FIS 389/94 and FIS 475/92.

INTERACTIONS BETWEEN THE TUBULOGLOMERULAR FEEDBACK MECHANISM AND THE MYOGENIC MECHANISM IN THE AUTORREGULATION OF RENAL BLOOD FLOW AND GLOMERULAR FILTRATION RATE. S7 1

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The intrinsic ability of the kidney to autoregulate renal blood flow (RBF) and glomerular filtration rate (GFR) in response to changes in renal arterial pressure over a wide range from about 70 mmHg to above 180 mmHg has been recognized for many years. The dynamic responses and the coupled autoregulation of RBF and GFR demonstrate the presence of a highly efficient negative feedback control mechanism that regulates afferent arteriolar resistance to levels needed to maintain the filtered load at the glomerulus. There is evidence that at least part of the autoregulatory response is mediated by a myogenic mechanism that is shared in common with other microcirculatory beds. However, renal autoregulation in the kidney is also mediated by the tubuloglomerular feedback (TGF) mechanism that serves as a link between the macula densa segment of the ascending loop of Henle and the vascular pole of the glomerulus. Under physiological conditions, there is a characteristic relationship between flow past the macula densa and the afferent arteriolar resistance which contributes to the autoregulation of renal blood flow and GFR in a variety of circumstances. When the TGF mechanism is disrupted, the efficiency of autoregulation of renal blood, glomerular filtration rate and glomerular pressure is reduced but persists, demonstrating that the myogenic mechanism alone can mediate partial autoregulatory responses.

Recent mathematical models have incorporated both the myogenic mechanism and the tubuloglomerular feedback mechanism to simulate high efficiency autoregulation. The input signal of the TGF mechanism involves the sensing of a downstream function of GFR and the output signal is to the vascular smooth muscle of the glomerular arterioles. The myogenic control subsystem responds directly to increases in wall tension with increases in vascular smooth muscle tone. Comparison of the model autoregulatory responses when the sensitivity of the TGF mechanism is varied indicates that a reduced TGF sensitivity will diminish renal blood flow autoregulatory efficiency. Increased ANG II levels result in increases in maximum TGF-mediated afferent arteriolar resistance responses while blockade of ANG II receptors diminishes TGF responsiveness suggesting that renal autoregulatory efficiency should be altered in parallel. In contrast, experimental studies have demonstrated that pharmacologic agents that reduce the sensitivity of the TGF mechanism do not diminish renal autoregulatory efficiency, but rather lead to a change in the absolute RBF at which autoregulation occurs.

These differences between the experimental data and the predicted results based on available mathematical models suggest complex overlapping interactions on the same vascular structure of the TGF mechanism and the myogenic mechanism such that there is redundancy of actions on the preglomerular vascular smooth muscle rather than additive effects. Consequently, it is possible that when the influence of the TGF mechanism on renal autoregulatory responsiveness is blocked or eliminated, the myogenic mechanism assumes a greater role than when the two mechanisms are operating in concert.

S7 2 TECHNIQUES FOR THE CONSTRUCTION OF ANALYTICAL CAUSAL MODELS OF PHYSIOLOGICAL SYSTEMS. L. Roa. Grupo de Ingeniería Biomédica, E. S. Ingenieros, U. S. 41012 Sevilla (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 44, 1997.

A common thread through all activities in the area of Biomedical Engineering is the requirement to understand the dynamic behaviour of systems with feedback properties. The importance of this need has been underscored by the addition of chapters on dynamic system theory to well-established textbooks on physiology[1].

Energy flows in many media (thermal, electrical, etc.) are governed by a common set of physical laws, although widely differing notations and conventions often obscure this fact. Paynter's tetrahedron of state[2] has helped to provide a unified view of such energetic systems. Here we present an extension Paynter's concepts to physiological systems.

Because the dynamics of energetic systems are due to the dissipation and storage of energy, it is natural to construct a system model in terms of variables that describe these actions. The variables that describe a system's condition or state are its state variables.

In general, the number of state variables required equals the number of energy storage compartments in the system. We usually express the relation for each compartment as a differential equation. We obtain a set coupled first-order equations for high-order systems. These equations are the state equations.

The stages to be followed for the construction of a model with our techniques can be summarised in two stages: a description by means of an orientated graph of the causal effect relationships between the different elements (or subsystem) which form the system; and, a formal description by means of a mathematical language (a mathematical model) of the established relationships of the previous stage.

The developed criteria to validate our models are: a) the dynamic behaviour obtained having simulated physiological situations cannot go against any experimentally or clinically proven fact and b) the model must be capable of reproducing observed behaviours as much for experimental as for clinical conditions.

The mathematical models built with these techniques have been used in different circumstances: for the teaching of physiological aspects of the control body fluid distribution or gas transport system in the human being, the analysis of the pulmonary capillary dynamics, or the analysis of the myocardial energetic metabolism in normal and ischemic situations among other aspects.

Beyond the specific application, we stress the role of mathematical model in the study of physiological control systems in general. In addition the study by simulation of variables which are not clinically observable promotes the comprehension of complicated systems and leads to the formulation of new hypotheses and/or to the design of new experiments.

References

1. A. C. Guyton and J. E. Hall, "Textbook of Medical Physiology", (9th ed.). W. B. Saunders Company, Philadelphia, 1996.

2. H. M. Paynter, "Analysis and Design of Engineering Systems", MIT Press, Cambridge, 1961.

USING MATHEMATICAL MODELS TO BETTER UNDERSTAND INTEGRATIVE PHYSIOLOGY. S7 3

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The traditional scientific approach to biomedical research is to seek simple answers to unsolved physiological problems. But there is no guarantee that correct answers are simple and, in fact, there is considerable evidence that many correct answers involve rather complex interactions. In these instances, theories can be expressed as mathematical models and computer-based simulations can be used to assist the more traditional research methods. The resultant simulations help to identify those experimental observations that support the theory (and those that do not, of course). If appropriate numerical tools are available, a mathematical model can be as complicated as reality dictates and the result will still be a meaningful one.

As an example, chronic hypertension is often characterized by elevated arterial pressure, normal cardiac output, elevated vascular resistance, normal body fluids and a suspected defect in kidney function. Mathematical models (*Circ. Res.*, 35:159, 1974) have been used to suggest that chronic hypertension may be caused by a primary defect in renal salt-and-water excretion, subtle salt-and-water retention and a peripheral vascular vasoconstrictor response to metabolic overperfusion. This concept can be extended to specific pathologies and even to the hemodynamics of growth.

It is not clear how many unanswered physiological questions might benefit from the use of complex theories. Two are mentioned here as possible candidates. Arterial hypoxia stimulates needed erythropoietin secretion by the kidney suggesting that blood oxygen tension is being monitored by the secreting cells. But anemia is at least as good as, and probably far better, at stimulating renal erythropoietin secretion in the absence of arterial hypoxia. This suggests that the processes that send a signal to the secreting cells may indeed be complicated; loss of hemoglobin may create a diffusion barrier near the secreting cells, for instance. A second example involves the renal response to changes in sodium intake. The kidney responds to decreased dietary intake by increasing renin secretion and decreasing sodium excretion to quickly reestablish sodium balance; the cause of this response is not obvious. It may be that small changes in tubular sodium flow are amplified into large changes in renin synthesis and secretion by positive juxtamedullary feedback. A mathematical model supports this concept.

The following may sometimes be important features of physiological control: long-term, high-gain negative feedback (showing little observable error signal), non-linear responses (possibly having a narrow response range), and low-gain positive feedback (requiring only a small stimulus). Traditionally, mathematical modeling has made little contribution to biomedical science because computers have not been readily available and the user-computer interface has been formidable. Modern numerical methods and desktop computers have removed these traditional barriers to using mathematical modeling as a scientific tool. (Supported by NIH-HL 51971).

S7 4 MODELLING THE THERMOREGULATORY SYSTEM. E. R. Nadel. The John B. Pierce Laboratory and Yale University School of Medicine, New Haven, CT 06519 (USA). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 46, 1997.

The thermoregulatory system is perhaps the body's most well understood and most thoroughly described regulatory system because of its relative simplicity. Heat transfer within the body occurs primarily by convection in blood. Heat transfer between the body and the environment occurs by convection, radiation and evaporation. Over the past two decades it has become clear that the body has in different loci specialized receptors that are sensitive to changes in temperature and participate in the directing of organ system responses that modulate the rates of heat transfer within and from the body. The thermosensitive elements are thought to be free nerve endings that are in high concentration in the preoptic anterior hypothalamus and over the skin surface. The thermosensors of the skin provide the thermoregulatory center, also thought to reside within the hypothalamus, with information about ambient temperature, and also serve as an early warning system in conditions of rapidly changing ambient temperature. The thermosensors in the hypothalamus assess the absolute body core temperature and are especially important during exercise, which is one of the few conditions in which internal temperature varies suddenly. The primary effector organs that provide for adjustments in heat flux in humans are the smooth muscles that control the tone of cutaneous blood vessels, the sweat glands, and skeletal muscle. The ability to adjust cutaneous vasomotor tone provides the means to modulate the blood flow, and therefore heat flow from core to skin. Over most of the skin the blood flow is controlled by active vasodilation. The ability to place water for evaporation onto the skin surface is due to activation of eccrine sweat glands. The secretory segment is innervated by the sympathetic nervous system, with acetylcholine the neurotransmitter. It is thought that the rate of sweat gland secretion is proportional to the rate of nervous activation. The skeletal muscles serve as the organ of heat production; shivering contractions are involuntary and mediated via the thermoregulatory mechanism. The integration center for temperature regulation evaluates the thermal state of the body, compares the thermal state with an idealized thermal state and, if these are not the same, directs changes in efferent activity that act to restore this idealized state. This theoretical model is then similar to the negative feedback model that applies to many physiologic regulatory mechanisms.

DYNAMIC MODELS OF RENAL BLOOD FLOW AUTOREGULATION. N-H. Holstein-Rathlou, M. Barfred and E. Mosekilde. Department of Medical Physiology, The Panum Institute, DK-2200 Copenhagen N (Denmark). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 47, 1997. S7 5

Autoregulation is the process that minimizes changes in renal function caused by fluctuations in the arterial pressure. Experimental studies have demonstrated that each nephron is equipped with at least two control mechanisms that could contribute to autoregulation: the myogenic mechanism, and the tubuloglomerular feedback mechanism (TGF).

The myogenic mechanism is an intrinsic response of the smooth muscle cells of the afferent arteriole. It is widely accepted that the myogenic response acts to minimize changes in vessel wall tension. Thus, the regulated variable is therefore probably not flow itself but rather wall tension, flow being stabilized because of the adjustments needed to minimize changes in wall tension.

TGF is a nephronal feedback mechanism that stabilizes renal blood flow, GFR, and the tubular flow rate. The anatomical basis for TGF is the return of the tubule (the ascending limb of the loop of Henle (ALH)) to its own glomerulus. The macula densa, which is the sensor mechanism for the TGF, is a plaque of specialized epithelial cells in the wall of the ALH. It is localized at the site where the tubule establishes contact with the glomerulus. Because of a flow dependency of NaCl reabsorption in the ALH, a change in tubular flow rate, elicited for example by a change in the arterial pressure, will lead to a change in the NaCl concentration of the tubular fluid. This is sensed by the macula densa, and through unknown mechanisms results in a change in the hemodynamic resistance of the afferent arteriole.

The dynamic properties of the TGF system has been characterized in experimental studies in both normo- and hypertensive rats. In normotensive rats, TGF displays autonomous self-sustained regular oscillations, whereas in spontaneously hypertensive rats (SHR) highly irregular, "chaotic" fluctuations are present.

Several attempts have been made to formulate mathematical models of the TGF system that is able to reproduce both the regular oscillations, and the irregular fluctuations. However, in most cases the models have been successful in describing the regular oscillations, but have failed to reproduce the irregular fluctuations seen in the SHR. It has only been possible to achieve irregular (chaotic) fluctuations in TGF through nonlinear extensions of the equations that describe the afferent arteriole. Although physiologically justified, these extensions have lacked a firm experimental basis.

To overcome the shortcomings of the previous models, we have in the present work extended a model of the TGF mechanism with a detailed model of the response of the afferent arteriole. To examine the bifurcation structure of this highly complex model we have applied one- and two dimensional continuation techniques. The results show that a Hopf bifurcation leads the system to perform self-sustained regular oscillations if the feedback gain is sufficiently strong. If the feedback gain is increased further, a folded structure of overlapping sheets of period-doubling cascades appear, leading ultimately to the appearance of classical chaotic behavior.

In conclusion, by including a nonlinear model of the response of the afferent arteriole, we are able to demonstrate the presence of both regular oscillations and chaotic behavior in a model of the TGF system. The results confirm previous studies that have suggested that the dynamic characteristics of the afferent arteriole are crucial for the appearance of chaos. Now, however, the results are obtained in a model that has a more satisfactory physiological basis.

ROLE OF CEREBELLUM IN MOTOR LEARNING. J. M. Delgado-García. Laboratorio de Neurociencia, Facultad de Biología, Universidad de Sevilla, Avda Reina Mercedes, 6, Sevilla-41012 (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 49, 1997. S8 1

The cerebellum has been implied in the acquisition and/or performance of new motor skills. Particularly, the electroresponsive properties of cerebellar nuclear cells endow them with a background firing that is continuously modulated by excitatory inputs from collaterals of mossy and climbing fiber afferents and by inhibitory inputs from overlying Purkinje cells. We have analyzed the interplay of those synaptic inputs in the alert cat by recording the unitary activity of identified posterior interpositus neurons during the acquisition and extinction of eyelid conditioned responses. Tones or short, weak air puffs were used as a conditioned stimulus, while long, strong air puffs presented at different intervals (50-1000 ms) were used as the unconditioned stimulus. The electrical response of those neurons was also studied during spontaneous and experimentally-evoked reflex eyelid responses. Main results indicate that nuclear neurons fire in synchrony with (but not in advance to) spontaneous, reflex, and conditioned eyelid responses. Consequently, their activity should be related to the performance of ongoing eyelid movements, but not to their initiation. Mossy and climbing fiber activity at the eyelid cerebellar cortex microzone was never observed to precede the electrical activity of the orbicularis oculi muscle.

S8 2 BIOPHYSICAL AND BIOCHEMICAL SUBSTRATES OF ASSOCIATIVE MEMORY IN CRITICAL BRAIN LOCI. B. G. Schreurs and D. L. Alkon. Laboratory of Adaptive Systems, NINDS, NIH, Bethesda, MD 20892 (USA). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 50, 1997.

Quantitatively assessed behavioral indices of associative learning and memory have been traced to specific brain loci and correlated with biophysical and biochemical events in diverse model systems (e.g., mollusks, rats, and rabbits). The consistency of these biophysical and biochemical events implicates a molecular cascade that has been conserved during evolution. In particular, cellular analyses of associative learning (classical conditioning, spatial maze learning, olfactory discrimination) for example, in the type B photoreceptor of *Hermissenda crassicornis*, pyramidal cells of the rat and rabbit hippocampus, and Purkinje cells of the rabbit cerebellum have revealed a cascade of cellular and subcellular events during memory formation. This cascade involves (a) intracellular elevation of calcium and diacylglycerol; (b) translocation of PKC; (c) phosphorylation of *calexcitin* (formerly cp20, which binds calcium and GTP); (d) inactivation of potassium channels; (e) regulation of protein synthesis; and (f) rearrangement of synaptic terminal branches. In *Hermissenda*, pairings of light and rotation have been shown to result in elevations of intracellular calcium and diacylglycerol in the type B photoreceptor which produce a translocation of PKC to the cell membrane and a resultant reduction in potassium-ion flow. These pairings also result in phosphorylation of *calexcitin* which has been shown to cause inactivation of potassium currents. In rabbits following classical conditioning of the nictitating membrane response (NMR) to tone and facial stimulation, intracellular and voltage clamp experiments have shown there is a reduction in potassium currents localized to the CA1 pyramidal cells of the hippocampus. This same area has been shown to undergo long-term activation of PKC following learning. Intradendritic recordings in the rabbit cerebellum following NMR conditioning have shown that there are increases in membrane excitability localized to dendrites of Purkinje cells in lobule HVI. These Purkinje cells are concentrated in the anterior medial portion of the left lobe of lobule HVI. This localization of excitability suggests the existence of learning "microzones" within lobule HVI. The increase in membrane excitability found in lobule HVI can be mimicked in slices from naive subjects by application of the potassium channel antagonist 4-AP suggesting a role for a rapidly inactivating potassium channel previously implicated in learning studies of *Hermissenda*. The same increased membrane excitability seen in Purkinje cell dendrites from trained rabbits can also be mimicked in slices from naive subjects by the application of the 20-kDa protein, *calexcitin*. Studies of synaptic modifiability of Purkinje cells in these microzones show that the pairing-specific long-term depression (LTD) observed in naive and explicitly unpaired control subjects is occluded in subjects that have received classical conditioning. Measurements of synaptic thresholds indicates that this occlusion results from an increase rather than a decrease in synaptic excitability. Finally, a number of recent human brain imaging experiments have revealed considerable functional involvement of the cerebellar cortex in eyelid conditioning suggesting that the human cerebellum may be a locus for the same cascade of cellular and subcellular events observed in our model systems preparations.

MOLECULAR PROBES OF PROTEIN KINASE C AND RELATED MOLECULES AS TOOLS FOR IMAGING MEMORY. S8 3

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Protein kinase C's (PKC) are actually a family of enzymes, discovered in the late 1970's on the basis of their affinity for tumor-promoting phorbol esters. Subsequently it was discovered that PKC plays a crucial role in a wide variety of signal transduction events in all eukariotic cells ranging from yeast to human neurons. More particularly, PKC was implicated in the neuronal modifications underlying learning and memory in *Hermissenda* and later on in rabbit hippocampus.

Conventional techniques for imaging brain functional activity are typically based on the physiological relationship between energy metabolism and spike generation in neurons. These methods, however, have not proven effective for the imaging of engram formation in large brain networks probably because of their inherent bias towards "glucose-guzzling" inhibitory interneurons which fire at high tonic rates.

Our initial strategy was to use the molecular specificity of radioactively-tagged phorbol ester for membrane-associated (activated) PKC. Using a tritiated phorbol -12,13- dibutyrate we were able to demonstrate dynamic, learning-specific changes in the distribution of activated PKC during acquisition and retention of the nictitating membrane conditioned response in the hippocampi of rabbits. Image analysis of the activated PKC image in fields CA1 and CA3 suggested a movement of the engram into the basilar dendrites of the pyramidal cells. Follow up studies on rats in the Morris water maze and in olfactory learning tasks demonstrated the usefulness of the PKC imaging strategy in more complex behavioral contexts. The utility of PKC as a probe for imaging learning and memory was confirmed by other groups using immunohistochemistry, western blotting and behavioral pharmacology. A major disadvantage of the autoradiographic methodology however has been its poor temporal resolution.

Two new fluorescent approaches have been developed in order to provide spatio-temporal information on rapid shifts in PKC distribution within individual cells and networks of cells. These new techniques have been used to visualize the activation of PKC *in vivo* in sea urchin eggs, subsequent to fertilization and the activation of phospholipase A2 in hippocampal slices in response to muscarinic challenge. Using the sea urchin egg as a test system, we have visualized a sustained translocation of PKC from the cytoplasm to the cortical membrane which lasts over the entire course of the first cell cycle. The sea urchin imaging data was confirmed with subcellular fractionation and western blots and, more recently, by another group using the fluorescently-labeled PKC inhibitor, FIM-1. The hippocampal slice imaging data employed a fluorescent substrate, NBD-phosphotidyl choline. All of the imaging data demonstrated a very high degree of sub-cellular spatial resolution combined with temporal resolution in the range of seconds.

In conclusion, the strategy of directing molecular probes against biomolecules which play essential roles in the mnemonic process has proven to be of great utility in imaging memory formation in brain networks. New techniques which are not dependent on autoradiography hold great promise in enhancing our understanding of these processes, both in the spatial and temporal domains.

S8 4 ASSOCIATIVE LEARNING IN THE PANCREATIC β-CELL. A MODEL OF NO NEURAL MEMORY SYSTEM. J. V. Sánchez-Andrés. Depto. Fisiología, Inst. Neurociencias, Univ. Alicante, 03080 Alicante (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1). 52, 1997.

Associative mechanisms are frequently present in neural systems. It is broadly accepted that they can subserve several types of learning and memory processes. It is reasonable to expect such biological success as these mechanisms endow a given system with two relevant properties: predictive capacity and coincidence detection. Association implies the appropriate pairing of inputs on a given system. The system should have biochemical properties allowing to produce different responses in dependence on whether the stimuli are provided independently or properly paired. These biochemical properties will be responsible for determining the response duration, that would be expected to be related with the subserved physiological process. Under this scope, it is reasonable to expect different durations of the learned processes. Such mechanisms have been extensively explored in neural systems, but there is not any reason to assume that should limited to them. In fact, they can provide the basis for a sustained effect appearing exclusively after a system detects that two specific inputs, properly correlated, act on it. Despite the potential roles that associative mechanisms can play in both neural and no-neural systems, they have not been described in the last ones.

We have studied the existence of associative mechanisms in the pancreatic β-cell. These cells are, physiologically, grouped in the islets of Langerhans, and secrete insulin in response to glucose increasing levels. It is well known that β-cells are able to secrete insulin in a reflex manner under the effect of vagal stimulation. This stimulation is attained before meals in a fashion very like Pavlov's dog salivation. It is generally assumed that this reflex is in the base of what is called the pre-absorptive or cephalic phase of insulin secretion, responsible for producing an increased level of the hormone in blood just before food will be absorbed by the gut.

In our experiments, we have demonstrated that the administration of glucose paired with a cholinergic agent increases temporally (about 1 hour) the sensitivity of the to glucose. Both, glucose and the cholinergic drug are required, as the administration of one of them isolated does not induce the effect. Under this scope, the role of the cephalic phase of insulin secretion gets a particular relevance because implies the pairing of glucose with vagal stimulation, with the consequence of modifying the glucose sensitivity of the system just along the period in which the physiological requirements are going to be higher: food absorption.

This effect can be considered a case of cellular associative memory in a no-neural system, allowing to hypothesize that associative mechanisms can be generalized in biology. The physiological relevance of this proposal relies on the fact that systems showing associative mechanisms could have some of the properties classically identified in neural systems.

MELATONIN AS A FREE RADICAL SCAVENGER AND AS AN ANTIOXIDANT. S9 1
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Free radicals, because of an unpair electron in their outer orbital, are highly reactive and freely damage macromolecules. Many of these are derived from molecular oxygen (O_2) with the most toxic free radical (the hydroxyl radical or •OH) being formed as a result of the 3 electron reduction of O_2. Melatonin has been shown, in a cell-free system, to be an effective scavenger of the •OH and to synergize with other antioxidants, e.g., vitamin E, vitamin C and glutathione in scavenging the ABTS radical. Additionally, melatonin is reportedly an effective peroxyl radical scavenger (which is generated during the peroxidation of lipids) although we have not been able to confirm this in cell-free *in vitro* studies. Nevertheless, in studies which used tissues and whole animals melatonin was found to be highly effective in reducing the accumulation of lipid peroxidation products induced by administration of kainic acid, ferrous iron, and paraquat and during the exposure of animals to hyperbaric oxygen. In these studies the tissues used included liver, kidney, lung, retina, brain and lens. Besides melatonin's ability to reduce lipid peroxidation it has also been shown to be effective in reducing DNA damage due to free radical-generating chemicals, e.g., safrole and bacterial lipopolysaccharide, and by a physical agent, ionizing radiation. Additionally, oxidative damage and cell death in cultured neurons induced by Alzheimer's amyloid β protein have been shown to be reduced by melatonin and, in animals, neuromorphological changes induced by the drug MPTP which causes Parkinson signs are abated by melatonin. These observations provide indirect evidence of melatonin's free radical scavenging activity *in vivo*. Whether physiological levels of melatonin are sufficient to provide measurable antioxidant protection remains to be established.

S9 2 MELATONIN AS A CELL NEUROPROTECTOR: EXPERIMENTAL AND CLINICAL STUDIES. D. Acuña-Castroviejo, E. Crespo, M. Martín, M. Macías, G. Escames and F. Vives. Instituto de Biotecnología, Universidad de Granada, Granada (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 54, 1997.

Increasing body of evidence support a role for melatonin as a cell protector. Neuroprotection by melatonin seems to be the common final pathway for the indoleamine action in the central nervous system. Melatonin exerts a depressive influence on CNS excitability, displaying anticonvulsant, anxiolytic and analgesic properties. As a consequence of melatonin action on brain, a potentiation of the GABA-benzodiazepine receptor complex and an inhibition of glutamatergic (NMDA and kainate) receptors takes place. Melatonin does not bind to GABA, benzadiazepine or glutamate binding sites themselves. These changes in neurotransmitter activity after melatonin administration seem to reflect the antiexcitotoxic activity of this compound. Several *in vivo* and *in vitro* data support melatonin as a highly specific free radical scavenger. Electrophysiological and biochemical data suggest that the antioxidant melatonin activity is the basis for its GABA and glutamate neurotransmitter modulation: regulating mitochondrial enzyme activity and neurosteroids synthesis in the first case, and changing the redox status of the NMDA receptor in the second one. The antioxidant properties of melatonin are an efficient mechanism to counteract brain oxidative stress in order to avoid neuronal damage. The indoleamine prevents lipid peroxidation induced by the administration of several neurotoxins such as paraquat and bacterial LPS, and protects against brain damage induced by reperfusion after ischemia. Melatonin also inhibits the NOS activity and NO production. Recently, it was demonstrated that pretreatment with melatonin prevents the nigrostriatal degeneration induced by MPIP injection in mice. Paraquat and MPTP (and other neurotoxins) increase mitochondrial oxidative stress blocking the intramitochondrial complex I. We recently found that melatonin significantly potentiates the mitochondrial enzymatic complex IV and, in a lesser extent, the I and II-III complexes in rat brain and liver. These data may represent an important role for melatonin into the cell since, due to the oxidative metabolism, the mitochondria produces high amounts of free radicals. These free radicals must be neutralized in order to maintain the normal mitochondrial metabolism and thus, the cell life.

Consequently, the antioxidant properties of melatonin explain many of the effects of this indoleamine on brain (and other tissues) and represent a phylogenetically well-preserved mechanism for the cell defense against oxidative stress. Besides, through the recently characterized nuclear receptor of melatonin, this indoleamine is able to genomically regulate the expression of several enzymes involved in the redox equilibrium, such as the glutathione peroxidase. During brain hyperexcitability status (as in epilepsy) the free radical production increases. Melatonin administration may be useful to counteract these clinical pathologies, as we have recently reported.

NUCLEAR SIGNALLING BY MELATONIN. C. Carlberg. Clinique de Dermatologie, Hôpital Cantonal Universitaire, CH-1211 Geneva 14 (Switzerland). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 55, 1997. S9 3

The pineal gland hormone melatonin is a small, lipophilic molecule that easily passes biological membranes. It is therefore rather obvious that melatonin enters the nucleus and may act as a nuclear hormone like, e.g., estrogen, progesterone, 1α,25-dihydroxyvitamin D_3 or retinoids. For the latter hormones within the last decade nuclear receptors have been identified that show a common modular structure and form the nuclear receptor superfamily. Nuclear receptors are transcription factors, i.e., direct modulators of gene transcription. For the vast majority of the members of the nuclear receptor superfamily no ligand has yet been identified and they are therefore referred to as orphans. Two independent observations linked the orphan nuclear receptor RZR/ROR[1-3] to melatonin: 1) the brain-specific β-subtype of the receptor shows highest expression in the pineal gland[4] and 2) the pharmacological considerations on anti-arthritic thiazolidine diones, which are synthetic RZR/ROR agonists, lead to melatonin[5]. The pineal gland hormone was shown to be able to activate RZR/ROR mediated gene expression at low nanomolar concentrations and nuclear extracts of RZR/ROR-overexpressing cells showed specific binding to melatonin[4,6]. These indications made it very likely that RZR/ROR is a nuclear receptor for melatonin and its pharmacological profile resembles to that of the previously described receptor Mel_2[7]. The recently cloned membrane receptors for melatonin (Mel_1) show an up to 20-fold higher affinity for the hormone than RZR/ROR, but are exclusively expressed in the brain[8]. In contrast, the α-subtype of RZR/ROR is nearly ubiquitously expressed and may be the key to understand those actions of melatonin in the periphery that are not relatad to its radical scavenger function. Nuclear signalling by melatonin, i.e. direct activation of the transcription factor RZR/ROR, opens up a new perspective on the function of the hormone. At least a subgroup of all RZR/ROR responding genes are also melatonin responding genes. This was shown first for human 5-lipoxygenase, a key enzyme of allergic and inflammatory reactions, which is down-regulated in a RZR/ROR-dependent fashion by melatonin and contains a RZR/ROR binding site in its promoter region[9]. This suggests an anti-inflammatory role for the pineal gland hormone and may explain the potency of synthetic RZR/ROR agonists in a rat rheumatoid arthritis model. The potentially most exiting role of melatonin is its cell cycle regulatory function, which could be explained by the action of the most recently identified RZR/ROR/melatonin responding genes $p21^{WAF1/CIP1}$ (inhibitor of cyclindependent kinases)[10] and cyclin A.

1. Becker-André, M. *et al. Biochem. Biophys. Res. Commun.*, 194, 1371-1379 (1993).
2. Giguère, V., *et al. Genes & Dev.*, 8, 538-553 (1994).
3. Carlberg, C. *et al. Mol. Endocrinol.*, 8, 757-770 (1994).
4. Becker-André, M. *et al. J. Biol. Chem.*, 269, 28531-28534 (1994).
5. Missbach, M. *et al. J. Biol. Chem.*, 271, 13515-13522 (1996).
6. Wiesenberg, I. *et al. Nucleic Acids Res.*, 23, 327-333 (1995).
7. Dubocovich, M. L. *Trends Pharmacol. Sci.*, 16, 50-56 (1995).
8. Reppert, S. M. *et al. Trends Pharmacol. Sci.*, 17, 100-102 (1996).
9. Steinhilber, D. *et al. J. Biol. Chem.*, 270, 7037-7040 (1995).
10. Schräder, M. *et al. J. Biol. Chem.*, 271, 19732-19736 (1996).

S9 4 INHIBITING EFFECTS OF MELATONIN ON THE NITRIC OXIDE METABOLISM IN THE CENTRAL NERVOUS SYSTEM. J. M. Guerrero. Dept. of Medical Biochemistry and Molecular Biology, The University of Seville School of Medicine, Avda. Sánchez Pizjuán 4, 41009-Seville (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 56, 1997.

The pineal gland, and its mejor hormone melatonin, translates environmental lighting information into signals that modulate reproductive, adrenal and other neuroendocrine interactions as well as immune function. A considerable body of evidence has accumulated suggesting that the mammalian pineal is capable of influencing central nervous system (CNS) function (Miles, 1988 #51) and, specifically constitutive nitric oxide synthase (NOS) activity. Thus, it has been shown that rat cerebellar NOS activity is inhibited by physiological concentrations of melatonin. The inhibition is dose-dependent and is coupled to an inhibition of the cyclic GMP production activated by L-arginine. Results also showed that calmodulin appears to be involved in this process because increasing its presence in the incubation medium prevented the effect of melatonin on both NOS activity and cyclic GMP production. Moreover, polyacrylamide gel electrophoresis studies suggest that melatonin interacts with calmodulin and modifies the binding of the peptide to the synthetic NOS peptide encompassing the calmodulin-binding domain of constitutive NOS from rat cerebellum, the natural mechanism by which calmodulin activates cerebellar NOS. Nitric oxide (NO) is recognized as a neurotransmitter in the CNS, being considered a mediator of the excitatory neurotransmitter glutamate. Constitutive NOS also occurs in hypothalamic neurons referred to as NOergic neurons. These neurons stimulate the release of a number of polypeptides from the hypothalamus including luteinizing hormone-releasing hormone (LHRH). Norepinephrine induces LHRH release by stimulating NO release from NOergic neurons. Norepinephrine acts on $\alpha 1$ receptors on NOergic neurons leading to increases in intracellular free calcium. Calcium ions combine with calmodulin to activate the constitutive NOS and, consequently, the production of NO. NO diffuses into the LHRH terminal, where it activates cycloxygenase which catalyzes the synthesis of prostaglandin E_2 (PGE_2) from arachidonate. PGE_2 then activates adenylate cyclase to generate cyclic AMP, which in turn induces exocytosis of the LHRH secretory granules into hypophyseal portal capillaries. We have reported that melatonin inhibits NOS activity, not only in rat cerebellum, but also in rat hypothalamus and, therefore, the hormone might be involved in the regulation of PGE_2 and cyclic AMP production. Indeed, we have obtained results showing that physiological concentrations of melatonin also inhibit the norepinephrine-induced activation of PGE_2 and cyclic AMP production as well as LHRH release. The inhibitory effect of melatonin may be mediated by inhibiting NOS activity, since the stimulatory effect of sodium nitroprusside (SNP), an espontaneous generator of NO, cannot be prevented by the hormone. In fact, melatonin also inhibited NOS activity in hypothalamus. Results suggest the existence of a new or an ancillary means by which melatonin may regulate the physiology of the hypothalamus-pituitary unit.

ION EXCHANGERS MEDIATING EPITHELIAL NaCl TRANSPORT. P. S. Aronson, D. Biemesderfer, T. Wang and G. Giebisch. Dept. Medicine and Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT (USA). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 57, 1997. S10 1

We have used the mammalian proximal tubule as an experimental system for studying mechanisms of NaCl transport in a model leaky epithelium. In the early proximal tubule, reabsorption of $NaHCO_3$ from the glomerular filtrate occurs largely by apical membrane Na^+-H^+ exchange. We developed isoform-specific monoclonal and polyclonal antibodies to determine the NHE isoform(s) expressed on the brush border membrane of proximal tubule cells. We found that NHE1 and NHE4 are basolateral membrane isoforms whereas NHE3 is present on the apical membrane of proximal tubule cells. NHE3 is also present in a population of subapical intracellular vesicles, suggesting possible regulation by membrane trafficking. Because work from other laboratories indicates that NHE2 is also an apical membrane isoform, we performed additional studies to assess the contribution of NHE3 to brush border Na^+-H^+ exchange activity, and to evaluate whether stimuli that increase apical Na^+-H^+ exchange activity are associated with increased expression of NHE3 protein. The inhibitor sensitivity of Na^+-H^+ exchange in renal brush border vesicles is markedly dissimilar from those reported for NHE1 and NHE2 but is nearly identical to reported values for NHE3. Na^+-H^+ exchange activity in apical membrane vesicles isolated from rats with metabolic acidosis is increased compared to control rats, with no change in inhibitor sensitivity. NHE3 protein expression is higher in brush border membranes from acidotic compared to control rats. Increased expression of apical membrane Na^+-H^+ exchange activity in the proximal tubule during renal maturation, and its further enhancement by glucocorticoids, is also associated with increased NHE3 protein expression. These results strongly suggest that Na^+-H^+ exchange activity in renal brush border membranes is mediated by NHE3 under baseline conditions and during the up-regulation of Na^+-H^+ exchange activity associated with metabolic acidosis, renal maturation, and glucocorticoid administration.

As a consequence of preferential isosmotic $NaHCO_3$ reabsorption in the early proximal tubule, there is a fall in tubular fluid pH and HCO_3^- concentration, and a rise in Cl^- concentration. The outward Cl^- concentration gradient provides a passive driving force for paracellular Cl^- reabsorption in the late proximal tubule. Because the tight junction permeability is higher for Cl^- than HCO_3^-, a lumen-positive transtubular potential difference is generated that drives passive paracellular reabsorption of Na^+ as well. Nevertheless, evidence suggests that a significant additional component of NaCl reabsorption in the late proximal tubule is active and transcellular. In isolated membrane vesicles we identified Cl^--formate and Cl^--oxalate exchangers as possible mechanisms of uphill Cl^- entry across the apical membrane of proximal tubule cells. For steady state Cl^- absorption to occur by these mechanisms, formate and oxalate must recycle from lumen to cell. Recycling of formate from lumen to cell may occur by H^+-coupled formate transport and nonionic diffusion of formic acid in parallel with Na^+-H^+ exchange. Oxalate recycling from lumen to cell takes place by oxalate-sulfate exchange in parallel with Na^+-sulfate cotransport. Cl^- exit across the basolateral membrane is most likely mediated by Cl^- channels. Consistent with these models, we found that transcellular NaCl reabsorption in the proximal tubule is dependent on the presence of physiologic concentrations of formate and oxalate in the luminal and capillary perfusates. Interestingly, in metabolic acidosis the activities of apical membrane Cl—base exchangers are down-regulated in parallel with the aforementioned up-regulation of brush border membrane Na^+-H^+ exchange. Thus, differential regulation of apical membrane ion exchangers may provide a mechanism to regulate the relative rates of $NaHCO_3$ and NaCl reabsorption as needed to maintain acid-base and NaCl homeostasis.

S10 2 MOLECULAR PHYSIOLOGY OF NUCLEOSIDE TRANSPORTERS IN LIVER PARENCHYMAL CELLS. M. Pastor-Anglada, A. Felipe, F. J. Casado, M. Gómez-Angelats, B. del Santo. Department of Biochemistry and Molecular Biology. University of Barcelona. 08071 Barcelona (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 58, 1997.

Liver parenchymal cells, when induced to proliferate, up-regulate a whole set of plasma membrane transporters which may be involved in a variety of cell functions, such as osmoregulation or nutrient supply for anabolic purposes. Although the hepatocytes show a marked endogenous synthesis of nucleosides we investigated the routes for nucleoside uptake in this cell type and its likely relationship with cell proliferation. Na^+-dependent uridine transport was characterized in liver plasma membrane vesicles (LPMV) and shown to translocate 1 Na^+ per 1 molecule of uridine. This transport activity was sensitive to membrane potential and seemed to accept both purines and pyrimidines. Further data obtained using isolated and cultured hepatocytes were consistent with the occurrence of at least two separate Na^+-dependent transport agencies, with different affinities for purines and pyrimidines. In hepatocytes, but not in LPMV, a NBTI-insensitive Na^+-independent equilibrative transport system was detected. Concentrative nucleoside transport in liver parenchymal cells seems to be modulated by a variety of factors. Hypertrophic hepatocytes show a much higher transport activity for nucleosides than their normal controls. Furthermore, in the prereplicative phase of liver growth after partial hepatectomy, Na^+-dependent nucleoside transport is enhanced (a 3-fold change in Vmax without changes in Km) in a manner which seems to be stable, does not depend on the Na^+ transmembrane gradient and is consistent with the synthesis and insertion into the plasma membrane of new carrier molecules. Glucagon is able to transiently stimulate Na^+-dependent nucleoside uptake by a mechanism involving changes in membrane potential. Insulin enhances this transport activity either in cultured hepatocytes or in *in vivo* models (euglycemic hyperinsulinemic clamp) in a way which is consistent also with the synthesis of new carriers. Homology cloning using the cNT1 cDNA led us and others to identify a novel nucleoside carrier related cDNA (SPNT) which seems to code for a Na^+-dependent purine-preferring transporter in liver cells. cNT1 and SPNT are the first two members of a new gene family. Situations cited above leading to enhanced Na^+-dependent transport activity correlate with increased amounts of SPNT mRNA. Transformed (hepatoma cells) and undifferentiated (fetal) liver parenchymal cells show a much higher transport activity for nucleosides than adult quiescent hepatocytes, but most of this activity is accounted for by an equilibrative Na^+-independent transport activity, which is partially or even totally sensitive to NBTI. Na^+-dependent nucleoside transport in transformed and undifferentiated cells is low or even negligible and also correlates with SPNT mRNA amounts. A second isoform seems to be present in hepatocytes but its likely role in the regulation of nucleoside uptake into liver parenchymal cells remains to be established. We conclude, first, that the SPNT mRNA levels are regulated in liver parenchymal cells thus inducing changes in the Na^+-dependent component of nucleoside uptake in hepatocytes, and, second, that the expression of the SPNT gene may be a feature of fully differentiated liver parenchymal cells. [Supported by D.G.I.C.Y.T., F.I.S.. and F.A P.S. (TV3 Marathon against Cancer)].

AQUAPORINS: HOW WATER CROSS THE CELL MEMBRANE. M. Echevarría-Irusta. Dept. de Fisiología y Biología Animal, Fac. de Farmacia, Univ. de Sevilla, 41012 Sevilla (Spain).J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 59, 1997. S10 3

The movement of water across the cell membrane is a process that occurs continuously in all living cells, particularly in those cells, like certain epithelia, that are involved in large movement of water. Water crosses the cell membrane by solubility/diffusion in the lipid bilayer and/or through water channels, a family of membrane proteins recently recognized and known as aquaporins (AQPs). The cloning of AQP1 (red cells, renal proximal tubules, descending thin limbs and multiple tissues), established the beginning for the identification of multiple members of the AQPs family by homology cloning: AQP2, the water channel regulated by ADH present in the apical membrane of the principal cells of the renal collecting duct; AQP3, colon and basolateral membrane of renal collecting duct; AQP4, brain and renal tissues; AQP5, eye, oral glands and lung. Aquaporins have also been identified in amphibia, insects, *E. coli* and in plants, and the list is increasing. Several features characterized the aquaporins family: they are rather small proteins ranging in size from 269-323 aa, the hydropathy analysis of the cDNA sequences predict that these proteins have six putative transmembrane domains with five connecting loops and both termini located intracellularly, they share two highly conserved amino acid sequences (NPAVT and NPAR). We have identified AQP3 and used this protein as a model to study selectivity and regulation of the expression of aquaporins. The full length cDNA has 1.9 kb, with an open reading frame of 876 bp that encodes a protein of 292 amino acids (Mr, 31,431). Immunoblots of cell membrane fractions of rat kidney cortex and medulla, using an affinity purified antibody anti-AQP3, identify a band at 27 kDa and a broader band at 33-40 kDa (glycosilated form). Injection of the cRNA of AQP3 into *Xenopus* oocytes markedly increase (~ 15-fold) the osmotic water permeability (P_f) and also to a lesser extent the permeability to formamide, glycerol and urea. Mercurial reagents abolish the expressed P_f but did not alter the glycerol permeability. The energy of activation (E_a) for the P_f is 3 Kcal/mole, whereas the E_a for the permeability to formamide, glycerol and urea had a higher value. *In situ* hybridization, immunohistochemistry and immuno-electron microscopic studies revealed that AQP3 and AQP4-labeling is restricted to the basolateral domain of principal cells in the renal collecting duct. Northern analysis studies demonstrated that kidneys of rats dehydrated for 48 h had a 2-3 fold increase in abundance of mRNA encoding AQP3 compare to well-hydrated rats. Identical results were observed in Brattleboro rats which lack of ADH. Immunoblotting studies confirmed an increase in the levels of AQP3 protein as a result of water restriction, however levels of AQP4 were not altered by dehydration. These studies indicate that AQP3 constitute a regulated pathway for water exit across the basolateral membrane of the collecting duct. AQP3 may also serve as a transporter for small solutes. Future directions include further studies in the understanding of the genetic and biochemical mechanisms that regulate aquaporins expression and function, and identification of additional aquaporins.

S10 4 STRUCTURE/FUNCTION STUDIES OF EPITHELIAL ISOFORM Na/H EXCHANGERS. M. Donowitz, M. Tse, C. Yun, S. Nath, R. Kambadur, S. Levine, J. Yip, A. Janecki, L. M. Corrochana, A. A. Ilundain and S. Shirazi-Beechey*. Center for Epithelial Studies, Depts. Medicine and Physiology, Johns Hopkins Un Sch Med, Balt, MD., *Dept. of Pharmacology, U. Seville, (Spain) and Institute of Biologic Science, U. Wales, Aberystwyth, Wales (U.K.). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 60, 1997.

NHE2 and NHE3 are epithelial isoform Na/H exchangers. Protein kinases regulate these exchangers by a V_{max} mechanism, in contrast to the class of housekeeping isoforms which are regulated by changes in affinity for intracellular H^+ ions. Based on immunocytochemical studies, both NHE2 and NHE3 are present on the brush border of small intestinal and colonic epithelial cells. Several insights into the mechanism of their regulation have recently been discovered: 1) A method to separate the contribution of NHE2 and NHE3 to brush border Na/H exchange has been developed. Both NHE2 and NHE3 contribute equally to basal rabbit ileal Na/H exchange, but the increase in brush border Na/H exchange seen following glucocorticoid treatment is all NHNE3. In avian small intestine, again NHE2 and NHE3 contribute to basal Na/H exchange, but the increase in Na/H exchange caused by a low Na diet is due to an increase in NHE3 activity. In avian colon, NHE2 accounts for the majority of basal brush border Na/H exchange and all the increase in response to mineralocorticoids. 2) Protein kinase regulation of NHE3 is associated with a change in amount of plasma membrane NHE3 based on quantitation via cell surface biotinylation. Serum increases and phorbol ester decreases the amount of NHE3 on the surface. 3) Stimulation of NHE3 by FGF or serum and inhibition by phorbol esters is not associated with changes in phosphorylation of NHE3. This supports a role for associated regulatory proteins in protein kinase regulation of NHE3. 4) One associated regulatory protein involved in regulation of NHE3 is calmodulin (CaM). Under basal conditions, NHE3 is inhibited by CaM and this occurs via CaM kinase II dependent and independent mechanisms. This effect involves binding of CaM to the NHE3 C-terminus. The part of the NHE3 C-terminus which is involved in CaM regulation of Na/H exchange is also the domain involved in binding of CaM.

FACTORS AFFECTING BLOOD VOLUME REGULATION DURING AND AFTER EXERCISE. E. R. Nadel. The John B. Pierce Laboratory and Yale University School of Medicine, New Haven, CT 06519 (USA). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 61, 1997. S11 1

Blood volume regulation during and after exercise consists of three components.

1) The rapid phase of blood volume regulation involves the action of the low and high pressure baroreceptors acting primarily to regulate arterial blood pressure. Plasma volume falls during exercise because of an elevated capillary hydrostatic pressure, increasing the capillary filtration force gradient, and an elevated muscle tissue osmolality, decreasing the capillary reabsorption force gradient. Central circulating blood volume also falls during exercise because water is lost from the body by the evaporation of sweat, and this water is ultimately derived from all the body fluid compartments, including the blood. Unloading the low pressure baroreceptors, as occurs when venous return is reduced, elicits a compensatory increase in splanchnic and cutaneous vascular resistance, acting to shift blood centrally and therefore ensure an adequate cardiac output and oxygen delivery to the contracting muscles.

2) The intermediate phase of blood volume regulation during the first hours of recovery from exercise involves the action of osmoreceptors and low pressure baroreceptors on the release of the hormones arginine vasopressin and aldosterone, and their subsequent action. During the initial recovery period, plasma volume recovers primarily because of an increased plasma osmotic pressure, which is explained by a greater loss of water than of electrolytes during exercise. During the subsequent hours, plasma volume stability is due to an increased plasma albumin content, which is the result of an elevated lymph flow during muscular activity. An increased osmoreceptor firing rate stimulates release of vasopressin, which in turn causes an increase in thirst and a decrease in renal free water clearance, both contributing to the rehydration process when water is available for intake. A reduced cardiac preload accompanying the transient hypovolemia unloads the low pressure baroreceptors, and along with a reduced renal blood flow, increases plasma aldosterone concentration, which increases renal sodium reabsorption.

3) The slow phase of blood volume regulation, occurring over the 24 - 48 hours following intense exercise, involves an increase in plasma albumin content which serves to draw fluid into and expand plasma volume above the pre-exercise level. The mechanisms underlying plasma albumin expansion include an upregulation in hepatic albumin synthesis and a decrease in albumin escape rate from the intravascular compartment.

S11 2 METABOLIC AND HORMONAL MODIFICATIONS DURING AEROBIC EXERCISE. V. J. Fernández-Pastor and A. M. Diego-Acosta. Fisiología del Ejercicio. Dpto. de Fisiología, Facultad de Medicina, Universidad de Málaga, Málaga (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 62, 1997.

We did a descriptive study of a group of long-distance runners in order to clarify aspects concerning neuroendocrine mechanisms regulating organic adaptation to maximum effort, with special interest in the function of the growth hormone in fat metabolism and the possible use of ketone bodies as an alternative source of energy. A test designed on a treadmill with a gradient of 3 % and progressive increases in speed of 2 Km/h every 10 minutes, starting at 6 Km/h, and continuing until exhaustion. Masks were worn to enable the breath by breath measurement of expired gases and the subjects were monitored electrocardiographically using V5. For blood sample collection an antecubital vein was catheterized with a system enabling the replacement of the blood volume extracted by means of perfusion with physiological saline solution, and we evaluated the increasing concentration of hormones in the blood.

Growth hormone levels were higher in the control group at baseline ($p < 0.001$) and during exercise when the same workloads were compared, which may be due to a greater receptor sensitivity in the runners. The existence of an inverse lineal regression ($p < 0.01$) between the concentrations of GH and FFA during exercise indicates that GH might facilitate the use of FFA by the active muscle cell and the lineal regression between GH and glycerol ($p < 0.05$) may involve the GH as a hormone which could facilitate lipid mobilization during exercise.

The blood glucose levels of the control group did not vary during exercise though in the recovery period there was an increase ($p < 0.01$), possibly due to the braking and later drop in its use. This increase in glucose was accompanied by an insulin stimulus in this period which could again have facilitated the uptake of glucose by the cell. The levels of blood glucose in the runners increased at minute 20 of the exercise, coinciding with the increase in epinephrine and GH. This increase remained stable at concentrations approaching 1 g/l for at least 30 minutes, until the end of the exercise, when there was a regulation in the levels of blood glucose in the runners, possibly due to a drop in the use of glucose by the muscle and an increase in hepatic glucose production. The decrease in utilization may be regulated by the reduction in insulin concentration, the increase in GH and by the utilization of FFA by the muscle. The increased hepatic production of glucose may be favored by the increases in epinephrine and GH from minute 20 of the exercise.

Among the results obtained, it was seen that the growth hormone facilitates the mobilization of lipids from depot organs and favors the utilization of free fatty acids by the active muscle cell, and that the muscle employs acetyl-CoA originating from acetoacetate as an alternative metabolic pathway during maximum effort.

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THE PHYSIOLOGICAL ROLE OF POTASSIUM IN EXERCISE. J. Ribas. Departamento de Fisiología Médica y Biofísica. Facultad de Medicina, Universidad de Sevilla, 41009 Sevilla (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 63, 1997. S11 3

Exercise stresses most of the physiological system in the human body. Neuromuscular excitation, energy transference, oxidative and anti-oxidative processes, cardio-vascular performance, fluid conditions of the blood, immunity, thermoregulation, hydric and acid-base balance among others. Potassium ions play a striking role in the functional connection between neural excitation and energy availability underlying power delivery during muscular activity. Membrane excitability is depending on extracellular potassium concentration ($[K^+]_o$) and may be impaired by small changes in $[K^+]_o$. During muscle contraction the breakdown rate of ATP occurs in a mode proportional to the power of contraction that is controlled by the rate of neurotransmitter release at the neuromuscular junction and, subsequently by the generation rate of sarcolema action potentials. Hypothetically the higher muscle work the higher the ATP breakdown and the $[K^+]_o$ accumulation. Trained and untrained subjects were testing to ascertain whether the concentration of plasma potassium ($[K^+]_p$) increase depending on work load and training condition. The maximal values of $[K^+]_p$ in untrained subjects were close to that in trained ones. However trained subjects needed higher work loads to reach the maximal $[K^+]_p$ and showed a faster time course in the recovery of $[K^+]_p$ accumulation after the exercise. The increased $[K^+]_p$ during exercise is basic for controlling arterial pressure, breathing, coagulation and anti-oxidation in addition to muscular excitation.

S11 4 GLUCOSE AND GLYCOGEN REGULATION DURING EXERCISE: THE "CROSSOVER CONCEPT". G. A. Brooks. Dept. Human Biodynamics, University of California, Berkeley, CA 94720 (USA). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 64, 1997.

The "Crossover Concept" has been presented to provide a theoretical means by which one can understand the effects of exercise intensity and prior endurance training on the balance of carbohydrate (CHO) and lipid metabolism during sustained exercise. According to the "Crossover Concept", endurance training results in muscular biochemical adaptations that enhance lipid oxidation as well as decrease the sympathetic nervous system (SNS) responses to given submaximal exercise stresses. These adaptations promote lipid oxidation during mild to moderate intensity exercise. In contrast, increases in exercise intensity are conceived to increase contraction-induced muscle glycogenolysis, alter the pattern of fiber type recruitment and increase SNS activity. Therefore, the pattern of substrate utilization in an individual at any point in time depends upon the interaction between the exercise intensity induced responses (which increase CHO utilization) and the endurance training-induced responses (that promote lipid oxidation). The "Crossover Point" is identified as the power output at which energy from CHO-derived fuels predominates over energy from lipids with further increases in power eliciting a relative increment in CHO utilization and decrement in lipid oxidation. The contemporary literature contains data to indicate that after endurance training exercise at mild intensities ($\leq$ 45 % VO_2max) will be accomplished with lipid as the main substrate. In contrast, the literature also contains reports which are interpreted to indicate that during hard intensity exercise (approximately 75 % VO_2max) carbohydrate is the predominant substrate. Seen within the context of the "Crossover Concept", these apparently divergent results are, in fact, consistent as the "Crossover Point" occurs at 50-55 % of VO_2max, regardless of training state. Because in their training and competition, most athletes will perform at intensities which elicit more than 70-75 % of maximum aerobic power, they will be dependent upon carbohydrates for energy. However, during recovery from exercises which result in glycogen depletion, lipid will become the predominant fuel. Possible regulatory factors such as myocyte glucose uptake (GLUT 4 tr anslocation), glycogenolysis (phosphorylase activation), PFK and PDH regulation, regulation of mitochondrial fatty acid uptake (malonyl-CoA), and the regulations of mitochondrial respiration will be discussed.