REVISTA ESPAÑOLA DE FISIOLOGIA. 40, 53-62. 1984

Effect of Anesthesia on Rat Respiration. A Study in Decerebrated, Decerebrated-Anesthetized and Intact-Brain Preparations

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(Received on April 19, 1983)

S. A. RODRIGUEZ-MENDEZ, M. FERIA, R. BORGES and J. BOADA. *Effect of Anesthesia on Rat Respiration. A Study in Decerebrated, Decerebrated-Anesthetized and Intact-Brain Preparations.* Rev. esp. Fisiol., **40**, 53-62. 1984.

The time course of respiratory parameters and blood pressure was studied in decerebrated rats (DR), decerebrated rats treated with a combination of thiopental plus urethane (DAR), and intact brain rats anesthetized with the same combination (IBAR). Moreover, the respiratory sensitivity to a stimulating dose of amphetamine was tested in the three preparations. DR exhibited a spontaneous and steady increase of ventilation which was absent in DAR. A steady increase of ventilation was also observed in IBAR, although of a lesser intensity. Amphetamine induced a clear respiratory stimulation which was decreased by the administration of anesthetics. A tendency to hypotension was seen in all animals. Therefore, the respiratory instability and the decreased pharmacological response observed in the presence of anesthetics are important factors to be considered when interpreting results obtained in this kind of preparations.

Key words: Anesthesia, Respiration, Blood pressure, Thiopental plus urethane, Respiratory parameters.

During the last few years, anesthetized rats have been used to study the effect of drugs on respiration (5-7). A combination of thiopental plus urethane has been frequently used. However, it is well known that this kind of compounds may produce respiratory depression. As a consequence, the interpretation of data obtained under the influence of such agents could be rather misleading. In order to attain suitable information about this topic, in this study respiratory and

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circulatory changes were monitored in decerebrated rats, decerebrated-anesthetized rats and intact-brain anesthetized rats. The pharmacological sensitivity of these preparations was tested in independent animals by using amphetamine.

Materials and Methods

Male Wistar rats weighing 350-400 g were used. The animals were allocated in three groups.

Decerebrated rats (DR). Tracheotomy and bilateral ligation of the carotid arteries were performed in 12 rats under halothane anesthesia. The animals were placed in a David Kopf stereotaxic apparatus and two lateral craniotomies were carried out on parietal bones. After ligation of the sinus longitudinalis, this structure and the sutura longitudinalis were removed together. Under direct vision, midcollicular transection was performed and the brain tissue rostral to the section removed by suction. At this moment, halothane was withdrawn and the left carotid artery and the right jugular vein were catheterized.

Anesthetized-decerebrated rats (DAR). Twelve animals were prepared as indicated in a) but sodium thiopental (50 mg kg^{-1}, i.p.) was used instead of halothane as the induction agent; 45 min after its administration, urethane (375 mg kg^{-1}, i.v.) was given. This time was considered adequate to permit recovery from the thiopental anesthesia.

Intact brain anesthetized rats (IBAR). In 11 animals, sodium thiopental (50 mg kg^{-1}, i.p.) was used as the induction agent to allow tracheotomy and cannulation of the left carotid artery and right jugular vein. Forty five minutes later urethane (375 mg kg^{-1}, i.v.) was administered.

Three other groups comprised of 10 animals each were prepared as indicated above to study the effects of amphetamine. This compound (amphetamine hydrochloride, Sigma) was administered by i.v. route at the dose of 1 mg kg^{-1}. This dose had been found to produce a significant respiratory stimulation in previous studies (5).

Once prepared, the animals were placed into a whole-body plethysmograph to study respiration. The volume changes inside the plethysmograph, as well as the end-expiratory CO_2 concentration and the blood pressure, were continuosly recorded on a Gould Brush dynograph through a Statham PM-15 differential air pressure transducer, a Godart infra-red CO_2 analyzer, and a Statham P23Db pressure transducer, respectively. In decerebrated rats, one hour elapsed from the moment of decerebration to the start of the observations. All animals were given oxygen-enriched air to breathe except in the periods of CO_2 inhalation. This was carried out at 0, 15, 45, 90 and 120 minutes. At these times an over-flow (3 l/min) of a mixture of 5 % CO_2 in O_2 was given for three minutes around the tracheal cannula. Throughout the experiments the body temperature was monitored by means of a Panlab electronic rectal thermometer and maintained at a constant level of $37.0 \pm 0.1°$ C. The time course of the following parameters was studied for a two hour period: respiratory frequency (f), tidal volume (V_T), respiratory minute volume ($\dot{V}_E$), end-expiratory CO_2 concentration, CO_2 responsiveness (by plotting end-expiratory CO_2 concentrations against respiratory minute volumes at resting and under CO_2 stimulation), and mean blood pressure.

Hystopathological examination was performed in three animals belonging to DR group in order to detect pulmonary alterations as responsible mechanism of the respiratory changes observed in this group of animals.

Statistical analysis. Comparison between the values obtained for each time and those obtained at the start of the observations were carried out (Student's t test). Moreover, the three series were compared reciprocally (analysis of variance). In the case of the CO_2-responsiveness analysis of variance for factorial planes (2 × 2) was effected.

Results

At the start of the experiments the f values were similar in the three groups. During the first 30 min a parallel increase of f was observed; however, after 45 min the f time course differed in each group. Thus, a steady increase was observed in DR, whereas a stabilization was seen in IBAR. An earlier stabilization followed by a decrease of f was observed in DAR. From a statistical point of view, only the changes noted in DR and IBAR were significant as compared with the control values ($p < 0.05$ at 120 min for DR; $p < 0.05$ from the 60th min in IBAR). As revealed by the analysis of variance, a significant difference was observed between the three series at 120 min ($p < 0.01$). In contrast to the similitude exhibited by the initial values of f at the start of the experiments, the V_T values were markedly different. It was thus shown that, as compared with DR, the V_T of IBAR and DAR were lower and significantly different

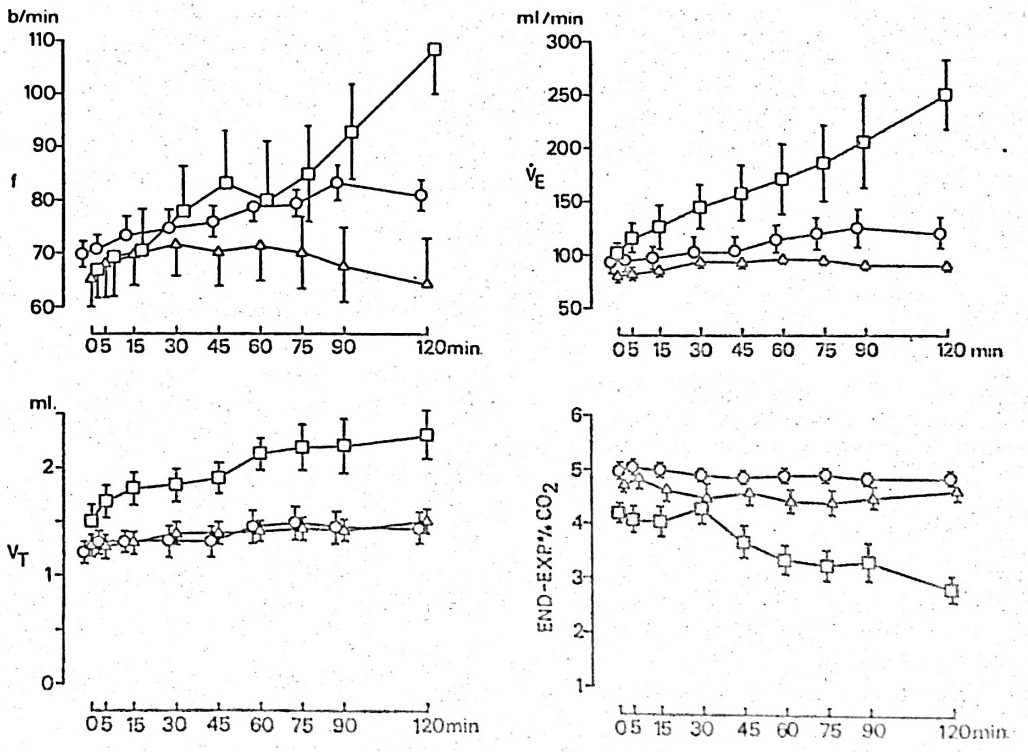

Fig. 1. *Time course of respiratory parameters in intact brain anesthetized rats (circles), decerebrated rats (squares) and decerebrated-anesthetized rats (triangles).*
f = respiratory frequency (breaths/min); end-exp. $\%CO_2$ = percent concentration of CO_2 in the end-expiratory air; $\dot{V}_E$ = minute volume (ml/min); V_T = tidal volume (ml).

Table I. *Time course of blood pressure.*

The values represent the mean ± S.E.M. of the per cent changes of mean b lood pressure calculated for each rat in relation to the control levels. Amph. = Animals treated with amphetamine. Mean control values were (mm Hg) DR, 132.28 ± 6.25; DAR, 80.88 ± 6.92; IBAR, 125.71 ± 6.80

	DR		DAR		IBAR	
Minutes	Control	Amph.	Control	Amph.	Control	Amph.
5	— 0.06 ± 1.45	— 1.32 ± 3.24	+ 2.47 ± 3.42	—18.01 ± 7.42	— 1.81 ± 1.72	—17.39 ± 2.04
15	— 5.98 ± 3.47	— 2.50 ± 4.27	+ 0.01 ± 2.86	—26.75 ± 4.85	— 4.92 ± 3.22	—21.93 ± 3.28
30	— 9.87 ± 4.62	— 4.24 ± 4.66	— 2.76 ± 3.66	—24.92 ± 5.95	—12.03 ± 3.75	—20.20 ± 3.08
45	—11.98 ± 3.47	— 7.57 ± 5.64	+ 3.69 ± 3.36	—17.07 ± 7.11	—15.66 ± 6.66	—16.17 ± 1.84
60	—20.10 ± 4.58	—17.06 ± 8.33	+ 7.91 ± 3.73	—15.34 ± 5.21	—14.40 ± 6.02	—10.19 ± 2.10
75	—16.86 ± 4.80	—17.00 ± 8.26	+ 5.35 ± 4.19	—12.13 ± 6.98	—10.36 ± 7.00	— 6.18 ± 2.32
90	—17.46 ± 4.08	—15.49 ± 6.68	+ 1.34 ± 4.49	—12.82 ± 7.05	—10.99 ± 5.86	— 3.73 ± 1.64
120	—15.59 ± 4.03	—18.75 ± 6.92	—0.94 ± 3.55	—11.17 ± 7.34	—10.17 ± 4.75	— 4.29 ± 1.72

($p < 0.05$). Concerning the time course of this parameter, a steady increase was observed in DR, although a statistically significant difference was not reached at any moment as compared with the values obtained at 0 time. The animals belonging to the other two groups showed a steady time course of V_T, although the actual values of this parameter were always lower than those observed in the DR group, as determined by analysis of variance. As might be expected from the f and V_T changes, $\dot{V}_E$ modifications were clearly present in DR. A steady increase of this parameter was seen in this group of animals, a statistically significant difference as compared with controls being found after 75 minutes up to the end of the experiment ($p < 0.05$-$p < 0.01$). Likewise, a significant difference was found between the DR group and the other two series from 75 minutes onwards. Concentration of CO_2 in the end-expiratory air was higher and more stable in both DAR and IBAR than in DR where a progressive decrease of this parameter was observed ($p < 0.05$ from the 60th minute). As compared with the other two series, this change was significantly different after 45 min ($p < 0.05$-$p < 0.01$). $\dot{V}_E$: CO_2-concentration straight lines (fig. 2) demonstrate a progressive increase of the CO_2-responsiveness, a statistically significant difference being reached at 120 minutes in the DR group but not in either DAR or IBAR. On the other hand, a displacement to the right and a reduction in the slope of $\dot{V}_E$-$\%CO_2$ straight lines in DAR and IBAR were observed as compared with DR ($p < 0.01$), as revealed by analysis of variance for factorial planes (2 × 2). The time course of blood pressure is presented in table I. A significant decrease of blood pressure was found in DAR from the start of the experiments. In the other two series there were no changes in blood pressure at 0 time but they showed a tendency toward hypotension.

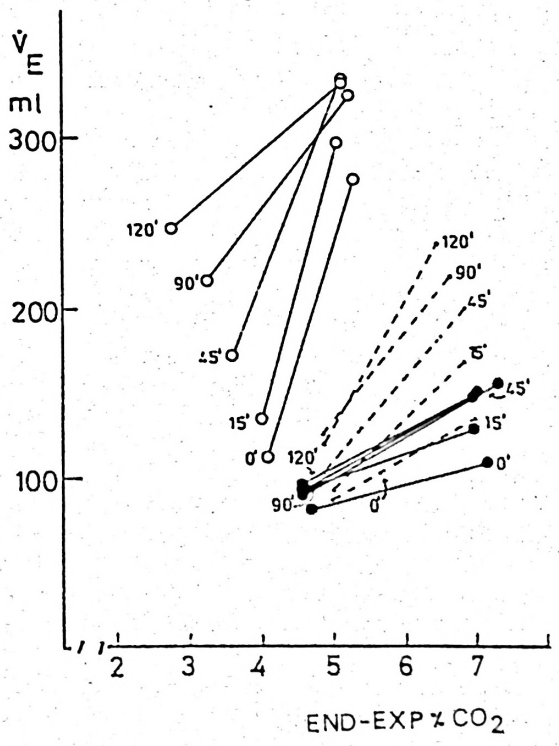

Fig. 2. *Time course of the relationship between end-expiratory % CO_2 and minute ventilation in decerebrated rats (open circles), decerebrated-anesthetized rats (closed circles) and intact brain anesthetized rats (dashed lines), at resting and under CO_2 stimulation.*

Tissular changes were not detected in the lungs of decerebrated rats at the end of the experiments.

Effect of amphetamine. Amphetamine caused an increase of f in the three groups of animals (fig. 3). This effect was particularly intense in DR ($p < 0.01$ from 30 min to 45 min). A progressive increase of V_T was observed in DR, whereas in the other two series there were no V_T changes except for a temporary rise seen at five minutes. $\dot{V}_E$ changes were similar to those observed in V_T although the increase seen at five minutes in DAR and IBAR was slightly more durable. CO_2-concentration

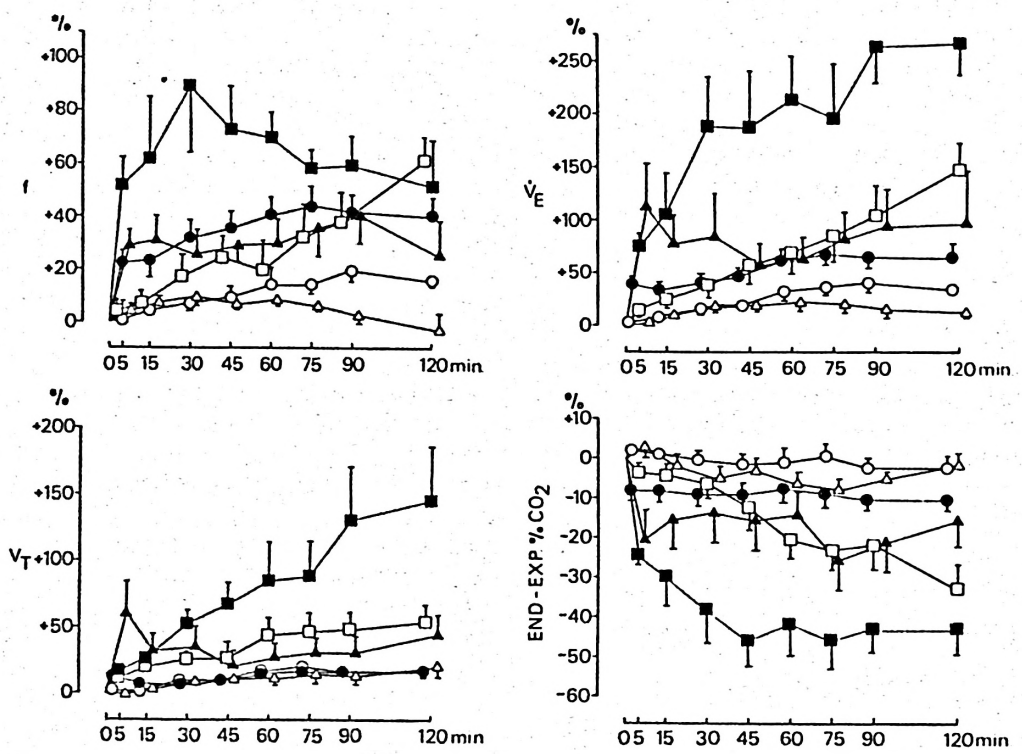

Fig. 3. *Time course of the percent changes induced by amphetamine on respiratory parameters.* Symbols as in figure 1 (open symbols: control animals; closed symbols: amphetamine, 1 mg kg^{-1}, i.v.). Control actual values were:

	DR	DAR	IBAR
f (b/min)	66.25±5.06	65.50±5.44	70.00±2.47
V_T (ml)	1.69±0.15	1.27±0.11	1.21±0.14
$\dot{V}_E$ (ml/min)	111.72±11.52	80.14±6.07	85.91±9.12
End-exp. % CO_2	4.21±0.18	4.72±0.16	4.98±0.16

in the end-expiratory air was modified according to the $\dot{V}_E$ changes. Thus a progressive decrease of this parameter was observed in DR whereas a transitory decrease was only seen initially in the other two series. As compared with DR, a displacement to the right and a reduction of the slope of the $\dot{V}_E$-%CO_2 straight lines were obtained in DAR and IBAR (fig. 4).

Discussion

The time course of the physiological parameters analyzed in the present study was clearly altered by all the experimental maneuvers employed. Thus, DR exhibited a steady increase of ventilation accompanied by a steady decrease of blood pressure these results confirming those obtained by other authors (5). The production mechanism of such a respiratory stimulation is unknown at the moment. Pulmonary embolism, which is a frequent complication after cranial surgery, would be ruled out in view of the results provided by the hystopathological study. By taking into consideration the steady increase in the CO_2-responsiveness and the respiratory

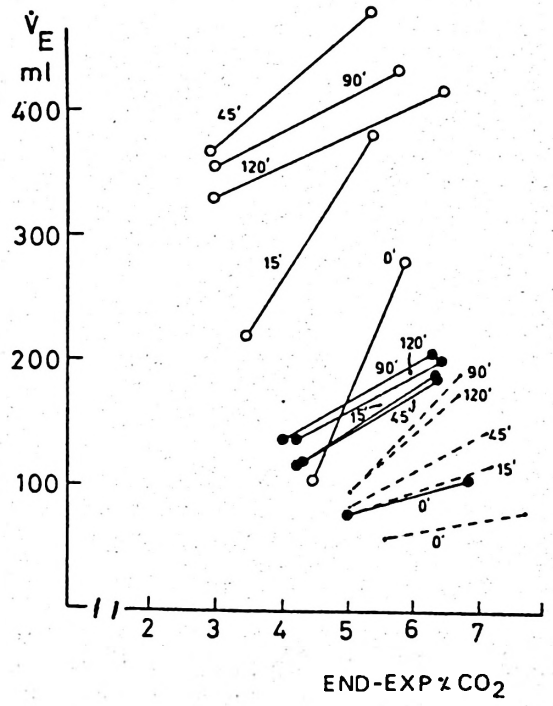

Fig. 4. *Effect of amphetamine (1 mg kg^{-1}, i.v.) on the time course of the same parameters shown in figure 2.*

stabilization produced by urethane anesthesia, it may be suggested that a central mechanism would be involved in this phenomenon. In contrast to our own observations and those reported by MEDIAVILLA *et al.* (5), a long lasting respiratory stability in decerebrated rats has been reported by SAPRU and KRIEGER (8), who moreover found that both pentobarbital and urethane did not produce V_T changes. Nevertheless, review of data presented by these authors reveals that the f, V_T, $\dot{V}_E$ and blood pressure values exhibited by their preparations were quite similar to those observed here by 120 min, that is, when the respiratory parameters had significantly increased as compared with 0 time values. On the other hand, report of the exact time their values were obtained was omitted. In these circumstances it is clear that a correct comparison with our own results would be difficult. Regarding the absence of respiratory depressant effects after the administration of some anesthetics, as demonstrated by the same authors (9), it must be taken into consideration that parallel controls were not employed in their study and, therefore, the lack of effects would actually correspond to depressant effects. Indeed, no differences were found between the pre-anesthetic and the post-anesthetic respiratory parameters in the anesthetized-decerebrated rats used in our own experiments. However, if the time courses of the respiratory changes in DR and DAR are compared, a respiratory depression (respiratory stabilization, apparently) is clearly seen in DAR. Therefore, in view of all these data it is evident that further studies are required in order to ascertain better characterization of the respiratory behavior of the decerebrated rat. Nonetheless, until definitive data are available, pharmacological tests performed in decerebrated rats without suitable parallel control must be interpreted with caution.

IBAR exhibited a slight and steady increase of ventilation. This was probably owing to the slow recovery from anesthesia, since the values of respiratory parameters observed by the end of the experiments were aproximately similar to those seen early on decerebrated rats. By this time these latter animals are presumably not yet under the effect of halothane.

The results obtained in both DAR and IBAR clearly indicate that the anesthetic association used here caused respiratory depressant effects. In this respect, the central depressant effect of barbiturates is wellknown and does not merit further comments. However, the fact that thiopental has a short lasting effect prompts the question of whether or not urethane was mainly responsible for the depression seen in these animals. The respiratory effects of urethane have been

studied only by a few authors. No change in CO_2-responsiveness after urethane administration was initially reported by WANG and NIMS (10). Likewise, CHVALOVA and PALECEK (3) found no changes in the breathing pattern of rats anesthetized with urethane. In the same way, no modifications in either spontaneous or neurotransmitter-evoked brain stem mononeuronal potentials in the rat were found by BRADLEY and DRAY (2), who proposed urethane as a satisfactory anesthetic agent. On the contrary, FLÓREZ and BORISON (4) demonstrated that this agent produced a reduction in V_T in the decerebrated cat accompanied by a decrease in the slope of the log $AL_{CO_2}/\dot{V}_E$ lines, mainly when the respiratory centres were electrically stimulated. Although this experimental technique was not performed in the present work, our results fully agree with those of the latter authors. In discussing the above mentioned discrepancies it is worthwhile to consider that, as indicated by BORISON (1), in the experiments carried out by WANG and NIMS (10) urethane actually produced a decrease in the resting ventilation. On the other hand, BRADLEY and DRAY (2) did not refer to the exact brain stem nuclei where the electrodes were placed; therefore, the possibility exists that the potentials of neurones belonging to the respiratory centres were not recorded. Concerning the observations of CHVALOVA and PALECEK (3), no conclusions could be drawn because quantitative data about pattern of breathing were not presented.

It is possible to conclude, therefore, that anesthesia by urethane in combination with thiopental, as the induction agent, may cause respiratory depression in the rat. The experiments in which the response to amphetamine was tested confirm this conclusion. Indeed, amphetamine produced respiratory stimulation in all tested animals, these results confirming those previously reported by MEDIAVILLA *et al.* (5), but its effect was clearly lower in the animals treated with the anesthetic combination. On the other hand, as compared with IBAR, the effect of amphetamine was more intense in DAR, at least for the first 30 min. This again shows the exquisite pharmacological sensitivity of decerebrated preparations. In fact, if one takes into consideration its durable respiratory stability, DAR would be proposed as an appropriate experimental model for pharmacological studies on respiration. However, it is not possible to assure that its sensitivity is maintained at a constant level throughout the experiments. Why did DAR not recover from anesthesia in a way similar to that seen in IBAR? A satisfactory answer is not available at the moment, but it is likely that an increased concentration of urethane in the central nervous system of DAR as compared with that of IBAR depending on the lessened amount of brain mass in the former may play a role in the occurrence of such a difference.

Blood pressure was also affected by the experimental maneuvers here used. All animals were prone to hypotension. The decrease of blood pressure was more intense in DAR than in the other groups, mainly because the pressure values of DAR were low at the start of the observations. The time courses of the blood pressure changes in the whole of animals roughly coincided with those described for respiratory modifications. Therefore, an interrelationship between both phenomena appears to exist. On the other hand, the hypotensive effect produced by amphetamine, which seems to be mediated by the activation of central alpha-adrenergic receptors (5), was more marked in DAR than in the other groups. Although the study of the blood pressure changes was not the aim of the present paper, the conclusion could be drawn that the anesthetic combination may also alter the circulatory responses to pharmacological agents.

In conclusion, in neuropharmacological studies carried out in rats submitted to thiopental-urethane anesthesia, the respiratory, and probably the circulatory, depressant effects caused by such an anesthetic combination must be taken into account in interpreting results obtained, emphasizing once more the need to include parallel control animals in the experimental design. On the other hand, decerebrated preparation in rats must be improved in order to achieve a suitable respiratory and circulatory stability.

Acknowledgments

Thanks are due to H. A. Argüelles for his technical assistance in the hystological lung examination.

Resumen

Se estudia en ratas el curso temporal de varios parámetros respiratorios y de la presión arterial en animales descerebrados, en descerebrados tratados con una combinación de tiopental y uretano, y con cerebro intacto anestesiados con dicha combinación, así como la sensibilidad respiratoria de estas preparaciones frente a una dosis estimulante de anfetamina. Las ratas descerebradas exhiben un aumento espontáneo y constante de la ventilación, el cual no se observa en las ratas descerebradas y anestesiadas. También se aprecia un aumento constante de la ventilación, aunque de menor intensidad, en las ratas con cerebro intacto y anestesiadas. La anfetamina produce una evidente estimulación respiratoria que disminuye en presencia de la combinación anestésica. En todos los animales existe tendencia a la hipotensión.

Por lo tanto, la inestabilidad respiratoria y la disminución de la respuesta farmacológica observadas en presencia de anestésicos, son importantes factores a considerar en la interpretación de los resultados obtenidos con este tipo de preparaciones.

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