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c-fos expression in the rat hypothalamic paraventricular nucleus induced by LiCl: descending projections to the dorsal vagal motor nucleus

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Anorexia inducing lithium chloride is believed to involve descending projections from hypothalamus to preganglionic autonomic output neurons. A multiple-labelling technique has presently been used to analyze the anatomical projections of lithium chloride sensitive neurons in the hypothalamus. Immunolabelling of c-fos was performed to stain neurons activated after LiCl administration, while neurons projecting toward vagal parasympathetic preganglionic levels were identified by injection of diamidino yellow in the dorsal motor nucleus of the vagus. Perikarya of descending neurons were mainly observed in the ventral and lateral areas of the paraventricular hypothalamic nucleus. In contrast, lithium chloride activated neurons were observed mainly in the magnocellular division of the paraventricular nucleus and supraoptic nucleus. Double-labelled neurons were not observed. These data provide evidence that lithium chloride sensitive neurons in the paraventricular nucleus are clearly different from those descending toward preganglionic vagal outflow neurons.

Key words: Paraventricular nucleus, Hypothalamus, Lithium chloride, c-fos, Diamidino yellow, Feeding behavior, Rat.

The paraventricular hypothalamic nucleus (PVN) is a major site of neurogenic and humoral control of the feeding behavior (13, 15). It has been established that while most PVN parvocellular neurons project to areas of central nervous system related to the regulation of autonomic functions (14, 18, 29), PVN magnocellular neurons produce vasopressin or

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oxytocin (3, 4, 23) and send their axons to the neurohypophysis (22, 28).

The analysis of the expression of c-fos protein has been shown to be useful for the study of the nerve cell physiological activity (6, 12, 19). Thus the c-fos protein expression in PVN neurons has been described after i.p. administration of lithium chloride (10, 31). Intraperitoneal administration of LiCl induces long lasting taste aversion (7, 2, 26) and alters feeding behaviour including pica (17), anorexia (5, 7) and emesis (7, 10). These actions have been suggested to be at least partly mediated via increased vagal output (1). Intraperitoneal administration of LiCl induces c-fos expression in the PVN and this nucleus via descending input toward preganglionic autonomic neurons of the dorsal vagal complex may influence vagal control of body energy homeostasis (14, 21).

In this report we have analyzed the topographic distribution of LiCl activated neurons in the PVN and their relation to autonomic outflow neurons projecting toward dorsal vagal complex.

Materials and Methods

Wistar rats weighing 350-450 g were used in all experiments. The animals (n = 9) were anesthetized with Ketamine 45 mg/kg and Diazepam 3 mg/kg, i.p. and were placed in a stereotaxic frame, with their heads ventrally flexed. The posterior atlantooccipital membrane was partially removed together with part of the occipital bone allowing visual inspection of the dorsal aspect of the lower medulla. Afterwards, 200-300 nl of Diamidino Yellow (DY) were bilaterally injected into the dorsomedial medulla in the dorsal motor nucleus of the vagus (DMX), 0.5 mm rostral to the calamus scriptorius, 0.5 mm to the midline and 1 mm under the dorsal surface, using a micropipette attached to a 1 ml Hamilton microsyringe. One week later, stimulated rats (n = 6) were injected i.p with a 3% LiCl solution (3 ml/kg). As control, animals (n = 3) were injected i.p. with a 4.14 % saline solution. Both solutions were physiologically hypertonic (10).

Two hours after injections, the animals were anaesthetized with a lethal dosis of barbiturate. Subsequently, the rats were perfused through the ascending aorta with 500 ml of 0.9 % phosphate-buffer saline followed by 500 ml of 4 % paraformaldehyde in 0.1 M phosphate-buffer solution as fixative. Brains were removed and immersed in the same fixative at room temperature during 2 hours. Afterwards, tissues were placed in a 30 % sucrose solution overnight. Brains were sliced into 50 µm frontal sections on a freezing microtome (Leitz) and processed for c-fos expression by using avidin-biotin-peroxidase method. Briefly, brain sections were stained with anti c-fos serum (genosys) diluted 1:3000 for 24 h at 4 °C followed by anti-sheep biotin-conjugated antibody (Vector Lab) for 1 h at room temperature diluted 1:200. Finally, tissues were developed in ABC Vectastain Kit (Vector Lab) for 2 h and in a VIP kit (Vector Lab) for 8 min. Hypothalamic nuclei were examined using an Olympus BX 60 fluorescence microscope (excitation wavelength: 360 nm) equipped with a camera lucida system. Anatomical landmarks of cerebral structures and injection sites were determined by counterstaining one of three sections. The PVN subdivision were named following the cytoarchitectonic criteria of SWANSON and KUYPERS (27), while other central nervous system structures were named according to PAXINOS and WATSON (20).

Results

The extent and placement of the injection sites were examined and included for further analysis if they were restricted to the surrounding area of DMX. Extension of injection sites were usually limited to 0.5-1.0 mm in the dorso-ventral direction, and 0.3-0.5 mm in the other directions. Retrogradely labelled perikarya were present throughout the rostrocaudal extension of the PVN and additional scattered cells were seen in the lateral hypothalamic area in all the experimental animals. Thus, retrogradely labelled neurons were found along the PVN (–1.3 to –2.4 from Bregma), with the highest density in the caudal two thirds, mainly in the medial and lateral parvocellular subnuclei. They were also detected in posterior magnocellular subnucleus and perifornical nucleus. In LiCl stimulated rats, c-fos stained neurons were found in most subnuclei of the PVN (fig. 1) and the supraoptic nucleus (SO). In contrast, fewer c-fos positive neurons were observed in the preoptic and posterior areas. In control rats, scattered weakly c-fos stained neurons were found in the PVN and SO. No diferences were observed in the rest of the hypothalamic structures analyzed.

In the PVN, c-fos stained neurons were found mostly in the rostral two thirds, mainly in the posterior magnocellular subnucleus (fig. 1B). Topographic distribution of retrogradely labelled neurons and that of c-fos positive neurons overlapped in the medial part of the PVN nucleus (-1.6 to -1.9 from Bregma), but clear subregional differences were observed. Thus c-fos immunoreacted neurons were located in the most dorsal and lateral parts, while retrogradely labelled neurons were located in the most ventral parts (fig. 1A and fig. 2). No DY/fos double-labelled cells were found.

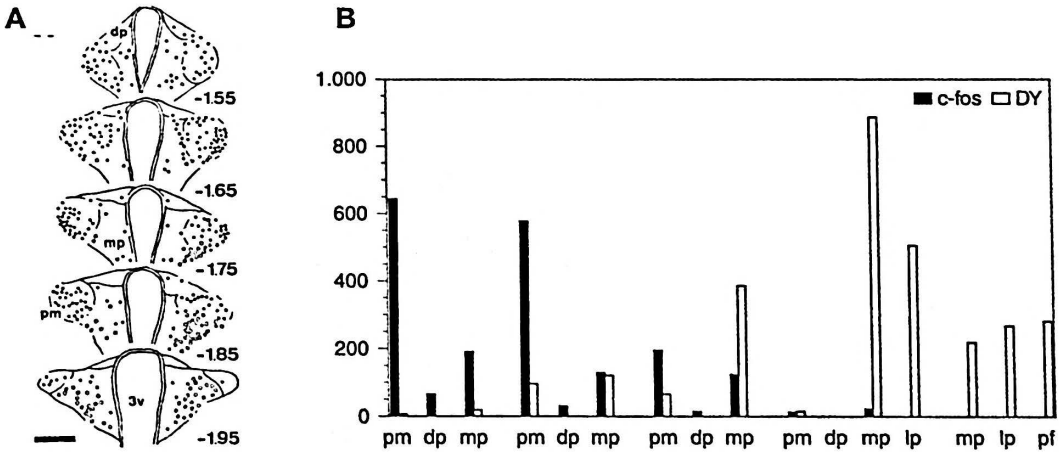

Fig. 1. *Distribution and number of c-fos and Diamidino Yellow (DY) labelled cells in the different subnuclei of the Paraventricular hypothalamic nucleus.*

A) Camera lucida drawing showing the distribution of Diamidino Yellow labelled hypothalamobulbar neurons (open circles) and c-fos LiCl sensitive neurons (black circles). Each symbol represents ten cells maximum. Bar = 400 µm. B). Number of DY and c-fos LiCl sensitive neurons in the different subnuclei of the PVN. Numbers indicate (in mm) the rostrocaudal position of the sections, zero being the bregma. dp, dorsal parvocellular subnucleus of the PVN; mp, medial parvocellular subnucleus of the PVN; pm, posterior magnocellular subnucleus of the PVN; lp, lateral parvocellular subnucleus of the PVN; 3V, 3rd ventricle.

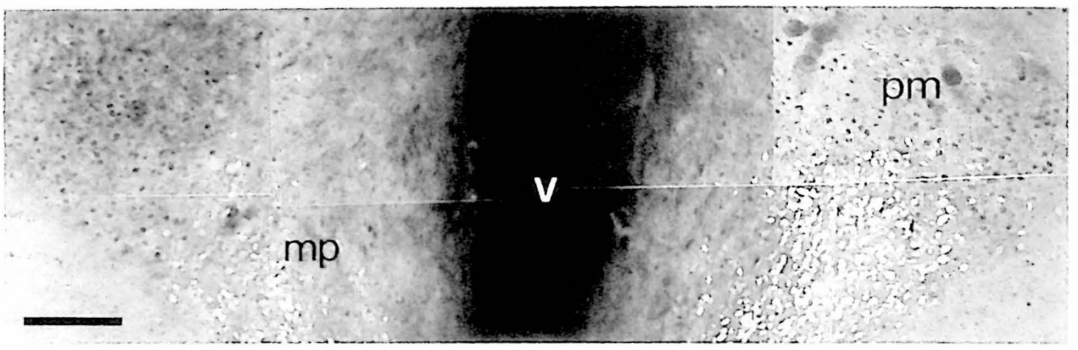

Fig. 2. *c-fos lithium chloride sensitive neurons and Diamidino Yellow labelled hypothalamobulbar neurons (DY) in the paraventricular nucleus of the hypothalamus (PVN), 1.85 mm caudal to the bregma.*
c-fos neurons are in the pm and DY neurons are in the mp. V, 3rd. ventricle; pm, posterior magnocellular ubnucleus of the PVN; mp, medial parvocellular subnucleus of the PVN; . Bar = 200 mm.

Discussion

The present findings on the distribution of c-fos stained cells after administration of LiCl are in agreement with previous reports (10, 31). Our results suggest that the PVN and SO c-fos stained cells are neurons which have been activated by LiCl injection. Furthermore, the combination of retrograde neuronal tract tracing and c-fos immunohistochemistry clearly demonstrates that despite striking overlapping of labelled cells, DY and c-fos were never co-localized in the PVN. Thus, the hypothesis that descending projections from hypothalamus toward autonomic preganglionic vagal neurons seems less likely to be involved in the response after LiCl administration.

Administration of LiCl causes an increase in oxytocin plasma levels (16). It is also well established that the PVN is involved in the release of neurohypophysial hormones (3, 4, 31). In fact oxytocinergic and vasopressinergic neurons are in the posterior magnocellular subnucleus of the PVN (8, 9, 23, 24), and LiCl activated cells are likely to be hypothalamo-pituitary neurons. However, we cannot rule out the possibility of a nervous response after administration of LiCl mediated by other PVN neurons different from descending neurons to autonomic preganglionic vagal neurons. In this sense, neurohypophysary neurons of the PVN have been described as projecting axons to autonomic centers of the central nervous system (28, 30).

Further research is needed in order to clarify whether LiCl activated neurons project to other levels of the central nervous system related to feeding behaviour and metabolism, such as the pontine Parabrachial Nucleus (26) or whether there are preganglionic sympathetic neurons in the spinal cord (2,15).

We conclude that in PVN in rats, LiCl activated neurons are located in the magnocellular portion dorsolaterally to descending neurons to autonomic preganglionic vagal neurons. Our immunolabelling approach showed a clear segregation between both types of neuronal populations. These results may contribute to a better understanding of the neural hypothalamic network involved in the autonomic control of feeding.

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En la anorexia provocada por el cloruro de litio pueden estar implicadas proyecciones descendentes desde el hipotálamo hacia neuronas preganglionares autónomas. En el presente estudio se lleva a cabo una técnica de marcaje múltiple analizando las proyecciones anatómicas de las neuronas sensibles al cloruro de litio en el hipotálamo. Las neuronas activadas tras la administración de ClLi se manifiestan mediante la realización de una inmunohistoquímica frente a la proteína c-fos, y las neuronas que proyectan hacia los niveles preganglionares parasimpáticos vagales se identifican mediante la inyección de amarillo de diamidino en el núcleo motor dorsal del vago. Se observan neuronas marcadas retrógradamente con el trazador fluorescente, principalmente en las áreas ventrales y laterales del núcleo paraventricular hipotalámico. Las neuronas activadas por el ClLi se localizan principalmente en la división magnocelular del núcleo paraventricular y en el núcleo supraóptico. No se observan neuronas doblemente marcadas. Estos resultados evidencian que las neuronas sensibles al ClLi son claramente diferentes a las que se proyectan hacia neuronas de niveles preganglionares vagales

Palabras clave: Núcleo paraventricular hipotalámico, Cloruro de litio, c-fos, Amarillo de diamidino, Conducta alimentaria, Rata.

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