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Effects of Pinealectomy and Sham-Surgery on the Area Postrema in Rats: A Quantitative Histological Study with Special Reference to Capillaries and Neuronal Cell Nuclei

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Summary. **This study aimed to clarify the effects of pinealectomy and sham-surgery on the area postrema (AP) by quantitative histological methods. Male, Wistar rats of normal (NO), sham-operated (SX), and pinealectomized (PX) groups were used in the late dark phase at 7 weeks of age. Consecutive frontal sections including the AP were stained with hematoxylin and eosin, and immunostained using PGP 9.5 for neurons, or GFAP or vimentin for glial cells. Consecutive sections of the AP were separated into five portions starting from the point of the central canal opening to the fourth ventricle in the caudal direction, and used for measurements. Mean cross-sectional areas of capillaries showed a lower value in the SX group than in the other two groups (vs NO, $P<0.005$; vs PX, $P<0.03$). In addition, the frequency distributions of the nuclear diameters of nerve cells showed different patterns among the three experimental groups ($P<0.01$), the frequency of large nuclei being higher in the SX group than in the other two groups. Possible mechanisms of the effects of sham-pinealectomy and pinealectomy and significance of the pineal-AP relation are discussed. The results of this study indicate that stuctural changes in the AP can be induced by intracranial surgery, suggesting certain pineal involvement in these changes.**

The area postrema (AP), one of the paraventricular organs, lies between the cerebrospinal fluid (CSF) compartment and the vascular system and is functionally closely related to both. In rodents, it is embedded in the caudal wall of the ventricle, overlying the entrance of the central canal (LESLIE, 1986). Histologically, the AP is covered with an ependymal cell lining relatively permeable to the CSF in its ventricular wall and rich in capillaries lacking blood-brain barrier; its parenchyma consists of small neurons, glial cells, and glia-like cells (BRIZZEE and KLARA, 1984; LESLIE, 1986). The neural element of AP has been implicated in a wide range of functions such as arousal, control of food and water intake, and cardiovascular functions, as well as representing the chemoreceptive trigger zone for the vomiting response (BORISON, 1989; JOHNSON and LOEWY, 1990; FERGUSON, 1992). It may serve as a fine-tuning mechanism for the autonomic nervous system with a multisensor device (LESLIE, 1986).

The AP is anatomically and functionally closely related to the nucleus tractus solitarius (NTS) (SHAPIRO and MISELIS, 1985; LESLIE, 1986; BORISON, 1989) and adrenalin neurons in the medulla oblongata (HÖKFELT et al., 1988; SAAVEDRA, 1988; BORISON, 1989). These structures have been shown to play major roles in the control of cardiovascular functions and also to have direct and/or indirect neural connections with spinal preganglionic sympathetic neurons (STOLK et al., 1988; GUYENET, 1990; LOEWY, 1990; DAMPNEY, 1994; BLESSING, 1997).

Melatonin, a hormone of another circumventricular organ, the pineal gland, is released following the circadian rhythm. Its involvement in reproductive and autonomic nerve functions including actions on the cardiovascular system has been documented (QUAY, 1974; VOLLRATH, 1981; KACHI, 1987; YU and REITER, 1993). We have repeatedly reported on the effects of pinealectomy (PX) and sham-pinealectomy (SX) on the adrenomedullary chromaffin cells and their nerve endings (KACHI, 1987; KACHI et al., 1997, 1998) as well as preganglionic sympathetic neurons in the thoracic spinal cord (T8) (IRIE and KACHI, 1999). Recently, it has been reported that the melatonin receptor exists in the AP (WEAVER et al., 1989; WILLIAMS, 1989; LAITINEN et al., 1990), and melatonin

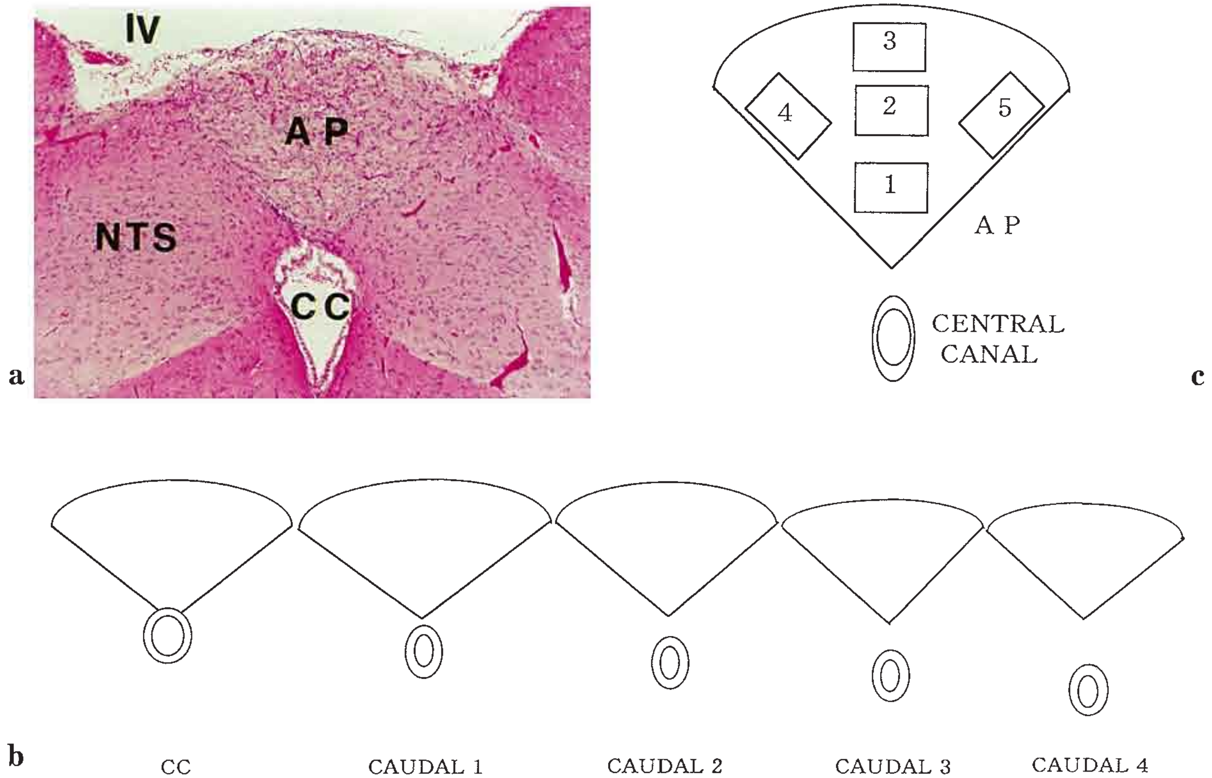

Fig. 1 a. The central canal portion of the area postrema (*AP*). It is contiguous to the central canal (*CC*) at this portion. *IV* fourth ventricle, *NTS* nucleus tractus solitarius. **b.** Regions in a cross-section of the AP selected for measurements. **c.** Portions of the AP and their relation to the central canal. NO rat, hematoxylin-eosin stain. ×57

increases the neuronal activity in the NTS (VITTE et al., 1989).

In the present study we investigated histologically those changes which might occur in the AP of pinealectomized (PX) rats, and noticed that microvessels in the AP were distributed more densely in PX than in sham-pinealectomized (SX) rats. Then we systematically investigated the effects of SX and PX on the cross-sectional area of capillaries and nuclear dimensions of constituent cells in the AP. Possible mechanisms of the effects of SX and PX and the significance of the pineal-AP relation are discussed in relation to the central control mechanisms of cerebral circulation and sympatho-adrenomedullary system.

MATERIALS AND METHODS

Wistar strain rats were purchased from a commercial source (Clea Co., Japan) at 21 days of age, and maintained in a windowless animal room with controlled temperature (22±2℃) and a 24 h light-dark cycle (lights on from 7AM-7PM). Food (MF; Oriental Co., Japan) and water were continuously available throughout the experiment. A total of 21 rats was divided into three experimental groups: normal (NO: intact, nonoperated, n=6), SX (n=7), and PX (n=8). Surgery was performed at 28 days of age. PX was done as described previously (QUAY, 1965). For SX, the cranial skull was opened using a dental drill under halothane anesthesia, a vertical incision about 2-4 mm in length was made from a point about 2 mm anterior from the sinus confluence in parallel with the mid sagittal sinus — about 1-2 mm apart, and fine tip fawceps were inserted but the pineal gland was neither extirpated nor disturbed. Then the skull was returned to its original position and the skin was closed by surgical bonding.

Animals were sacrificed from 6 AM to 7 AM at the age of ca. 49 days, i.e., three weeks after the surgery. Immediately after decapitation, the brain tissue in-

cluding the AP was fixed in Bouin's fluid for 24 h and dehydrated in a series of ethanol. After clarification with benzene, tissues were embedded in paraffin. Serial frontal sections were cut to a 7 μm thickness and stained with hematoxylin and eosin (Fig. 1a). For immunostaining, similar brain tissues of normal rats of 6-8 weeks of age (n=10 and several) were fixed in Bouin's or Zamboni's fluid for 12-24 h, embedded in paraffin or in O. C. T. compound (Miles Scientific Co., USA) and then frozen in liquid nitrogen, to be used for staining after sectioning.

Primary antibodies to glial fibrillary acidic protein (GFAP; DAKO Co., USA) and to vimentin (DAKO), both known as glial markers, were used at dilutions of 100× and 25×, respectively, and incubated for 12 h. Protein gene product (PGP 9.5; UCL Co., UK), a neuronal cell marker protein, was also used at a dilution of 100×. As the secondary antibody, a commercially available labeled streptavidin biotin kit (DAKO) was used. The antigen-antibody complex was visualized with a mixture of 0.02% 3,3'-diaminobenzidine tetrachloride and 0.01% H_2O_2 in 0.05 M Tris buffer, pH 7.6. The specificity of immunoreactions was checked by substitution of the primary antibody with non-immune rabbit serum. Following immunohistochemical staining, the sections were counterstained with methylgreen or hematoxylin and examined with a light microscope.

Quantitative methods

Measurement of cross-sectional area of capillaries

Using consecutive sections of the AP starting from the portion at which the central canal opens to the fourth ventricle and adjoins the AP closely, every fifth section was selected for a total of five. The first section, close to the central canal, was designated as the CC portion, and the following four sections as the 1st, 2nd, 3rd and 4th caudal portions, respectively. Thus the rostral end and the caudal end portions were not used for the measurements (Fig. 1b). In each section, cross-sectional areas of the AP and the capillaries and venules were measured using an image analyzer (IBAS, VIDAS; Karl Zeiss Co., Germany).

Measurement of the nuclear size of nerve cells

In each experimental group, five animals were used. The AP was divided into five regions (Fig.1c) in the CC and 2nd caudal portions. In each region, photomicrographs were taken at 250× and enlarged prints at 1000× were made. Using a dial caliper (TZ SK3; Kokuyo Co., Japan), the short and long diameters of nerve cells in the micrographs were measured, and the nuclear sizes were determined by calculating the means of two diameters. The frequency of nuclei over 7.5 μm in mean diameter was recorded.

Statistical methods

Analysis of variance, Student's t-test, Kraskal-Wallis test, and χ^2-test were used in the evaluation of statistically significant differences of nuclear sizes.

RESULTS

General observations

In hematoxylin-eosin-stained, consecutive sections, the AP was included in almost 60 sections in the rostro-caudal direction. In rats, the AP existed in the mid-dorsal area of the medulla oblongata and in the transitional part from the fourth ventricle to the central canal, and could be recognized as a single hill-like structure, showing a fan-like shape having a pivot at the ventral end in the frontal plane. The AP gradually became closer to the central canal when approaching the opening to the fourth ventricle, adjoining the slightly enlarged central canal at the CC portion. Only a few cross sections of capillaries were found at the end of the caudal portion. Their number increased gradually in the rostral direction and were abundant at the CC portion. At the end of the rostral portion, cross sections of large blood vessels were further increased and conspicuous. When comparing the three experimental groups, marked differences were seen not at either end portion but only in the mid portion between the CC and 4th caudal portions. Here the luminal sizes of vessels were obviously smaller in the SX group than in the other two groups (Fig. 2).

In immuno-stained sections using the anti-GFAP antibody, a small number of filamentous processes of glia cells appeared diffusely in the inner portion of the AP. In immuno-stained sections using the anti-vimentin antibody, immunopositive cells were slightly more numerous than the GFAP-positive cells, since vascular walls also showed positive reactions in the AP; other brain tissues were immunonegative except for the mid-sagittal line between the AP and the central canal, and ependymal cells lining the central canal and the fourth ventricle.

Immunostaining using the PGP 9.5 demonstrated the cytoplasm of neuronal elements in the AP. Their nuclei were round in shape, and the nuclear membrane was generally conspicuous and showed infoldings. Nucleoli were large, and chromatin presented a coarse and granular appearence. These morpho-

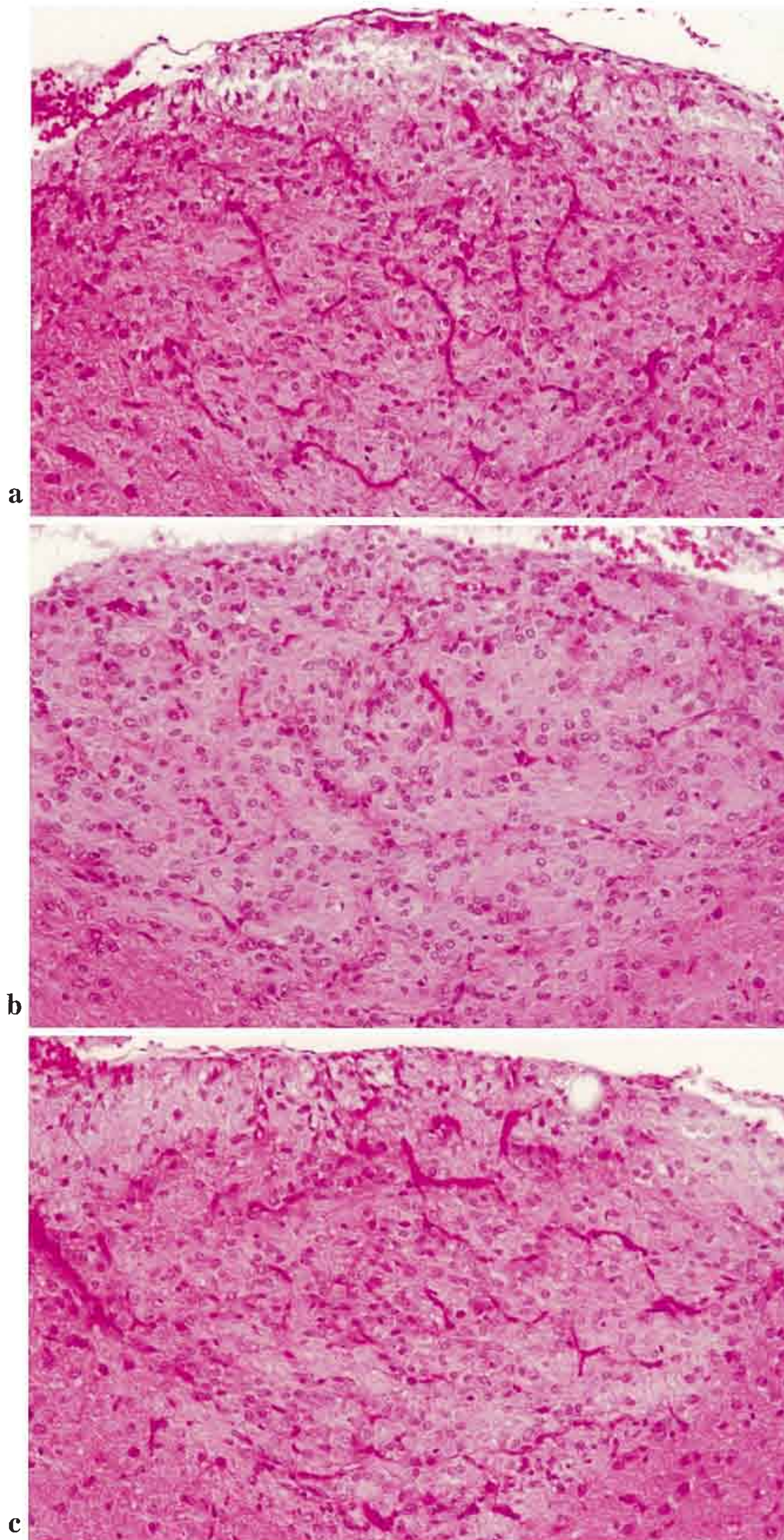

Fig. 2. Histological sections of the caudal two portions of the AP in the three experimental groups of rats. Hematoxylin-eosin stain. ×190.
a. Sections of capillaries containing red corpuscles are seen in abundance. NO rats. **b.** Sections of capillaries are seen much less frequently. SX rat. **c.** Sections of capillaries are seen relatively in abundance. PX rat.

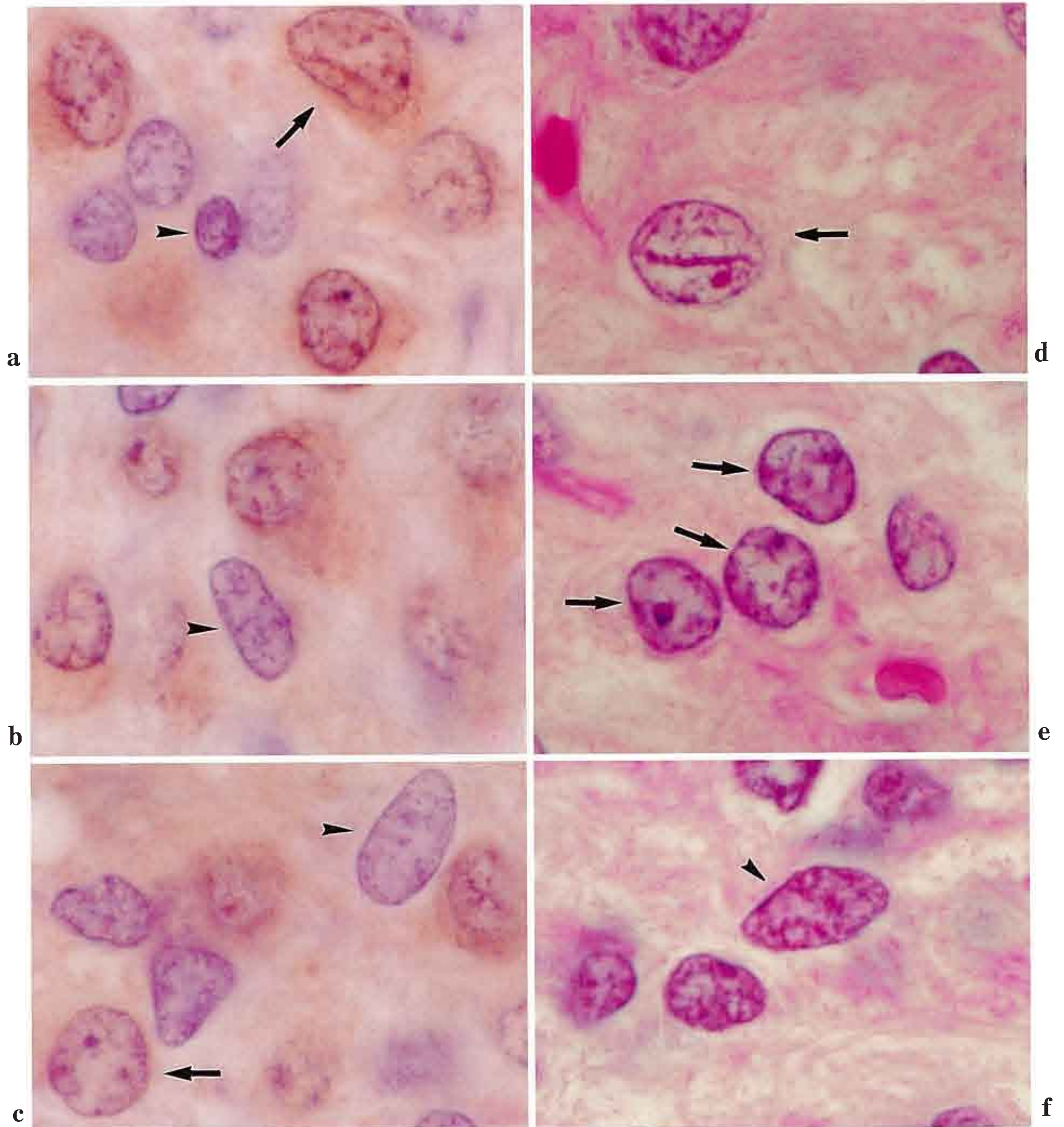

Fig. 3. Neural tissue cells immunostained for PGP 9.5 (**a,b,c**) and stained for hematoxylin-eosin (**d,e,f**) in the AP. ×1,900. **a.** An immunopositive neuronal cell (*arrow*) having a large nucleus and an immunonegative glial cell having a small dark nucleus (*arrowhead*) are seen. NO rat. **b.** An elongated nucleus of an immunonegative glial cell (*arrowhead*). NO rat. **c.** A spheroid nucleus of an immunopositive neuronal cell (*arrow*) and a large elongated nucleus of an immunonegative glial cell (*arrowhead*). NO rat. **d.** A nucleus of a neuronal cell (*arrow*) showing the nuclear membrane invagination. SX rat. **e.** Nuclei of neuronal cells (*arrows*). PX rat. **f.** An elongated nucleus of a glial cell (*arrowhead*). SX rat.

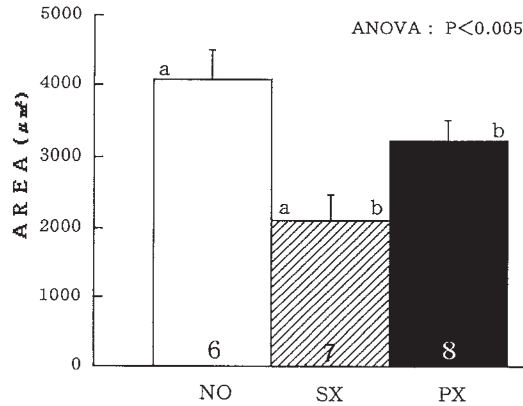

Fig. 4. Mean area of capillary sections. Numbers in bars represent numbers of animals. Mean ± SE. Student's t-test, a: $P<0.005$, b: $P<0.03$

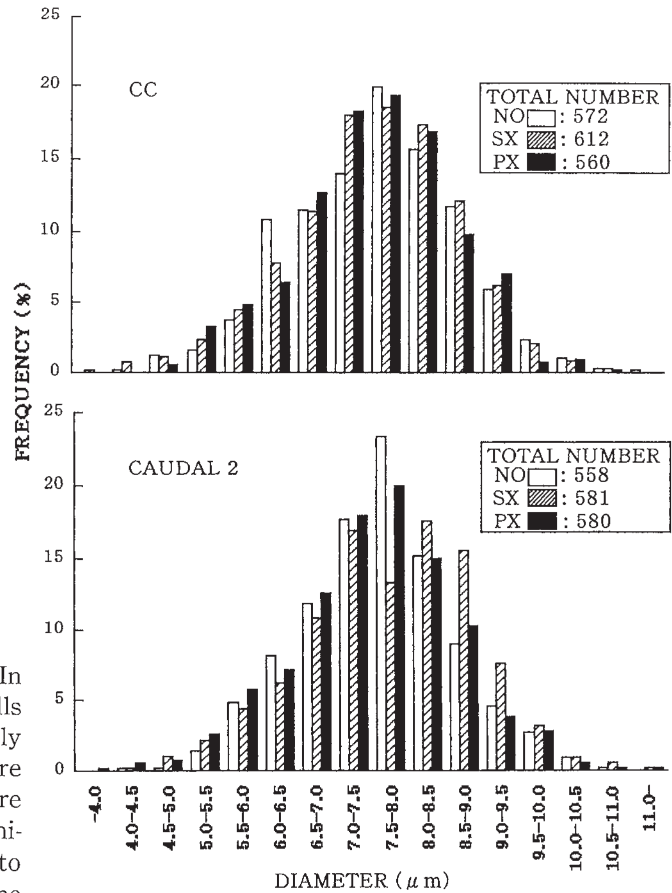

Fig. 5. Frequency distribution histogram of diameters of cell nuclei.

logical features were characteristic of nerve cells. In contrast, nuclei of PGP 9.5-immunonegative cells were variable in size and shape. Chromatin generally was distributed finely and diffusely; nucleoli were smaller in size. Dark nuclei varied in size and were round or elongated in shape. These nuclei were similar to those of glia cells. Thus, it was possible to differentiate nerve cells and neuroglial cells on the basis of the morphological characteristics of nuclei (Fig. 3).

Quantitative observations

Capillaries

The mean cross-sectional area of capillaries in the AP showed a significantly lower value in the SX group than in the NO and PX groups (ANOVA: $P<0.005$, Fig. 4), but the mean cross-sectional area of the AP itself and the mean number of cross sections of capillaries did not show a significant difference among the three groups. Similar comparisons were then performed among the five portions. Significant differences in the mean cross-sectional area of capillaries among the three experimental groups were seen in the CC, caudal 2nd and 3rd portions (ANOVA: $P<0.02$ in each portion), but not in the caudal 1st and 4th portions.

Nuclear diameter of neural tissue cells

In the CC and caudal 2nd portions, the nuclear size distribution of neural tissue cells in the AP is shown in Figure 5. No differences were seen in the mean nuclear diameter among the three experimental groups. However, while the NO group and the PX group showed the highest frequency between 7.5 and 8.0 μm in diameter, the SX group showed this between 8.0 and 8.5 μm in the caudal 2nd portion. The frequency of large nuclear profiles exhibited a higher value — which was statistically significant only in the caudal 2nd portion ($P<0.05$) — in the SX group than in the NO and PX groups (Fig. 6).

In these two portions, the frequency of nerve cell nuclei occurred at a much higher rate than that of neuroglial cell nuclei, the ratio of occurrence being 7–8 : 1. No difference in the nuclear diameter of nerve cells was seen among the three experimental groups in the CC and caudal 2nd portions. The frequency distribution of nuclear diameters of nerve cells is shown in Figure 7. The SX group had the lowest frequency of the shortest diameter range, and that of

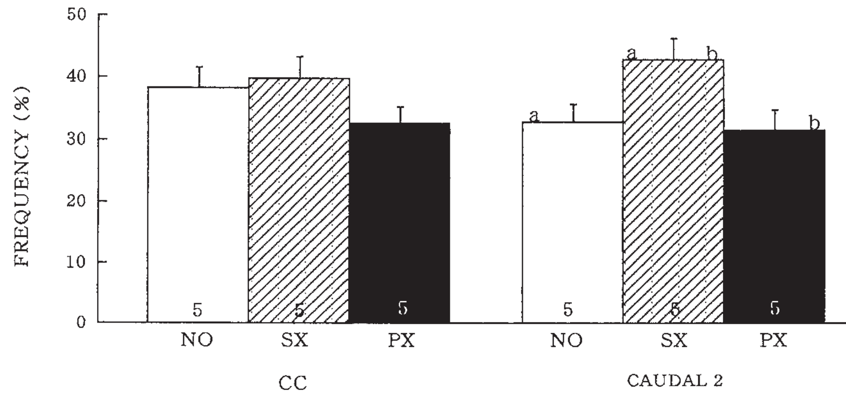

Fig. 6. Frequency distribution histogram of large nuclei (diameters ≧ 7.5 μm) in each experimental group. Numbers in bars represent numbers of animals. Mean ± SE. Student's t-test, a,b: P<0.05

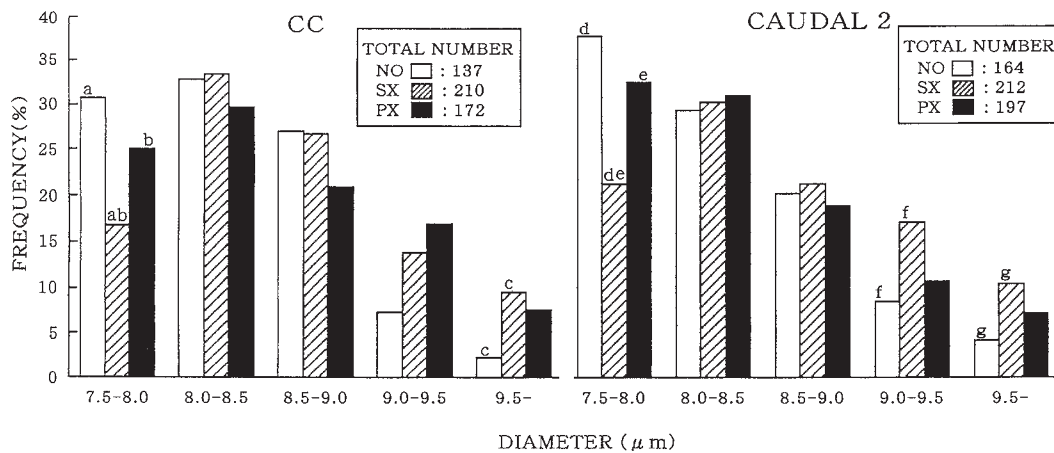

Fig. 7. Frequency distribution histogram of diameters of neuronal cell nuclei. Kraskall Wallis test, CC portion: P<0.01; caudal 2 portion: P<0.001. χ^2-test, a, c: P<0.01; b, e, f, g: P<0.05; d: P<0.001

its longer nuclear diameters often indicated higher values in both CC and caudal 2nd portions, especially compared with the NO group. The highest frequency was seen between 7.5 and 8.0 μm in diameter in the caudal 2nd portion in each of the NO and PX groups, but ranged between 8.0 and 8.5 μm in the SX group. Thus, different distribution patterns were recognized between the SX group and the NO group and between the SX group and the PX group, these differences being more marked in the caudal 2nd portion (P<0.001) than in the CC portion (P<0.01).

DISCUSSION

In this study, the mid portion of the AP starting from the CC portion and progressing toward the caudal direction — except for both the rostral and caudal ends — was used for measurements because our preliminary observations and other previous studies indicated that: 1) cross-sections of the rostral end of the AP are irregular in shape, 2) luminal sizes of microvessels in neither the rostral nor in the caudal end show apparent changes due to experimental treatment, and 3) the CC portion can be a constant

starting point in different animals and is rich in capillaries (GROSS, 1992). The mean cross-sectional areas of microvessels in the selected portions of the AP showed significantly lower values in the SX group than in the other two groups. There seems to be no reason to believe that the extent of surgical invasion is stronger in SX than in PX. It is likely, therefore, that SX influences the luminal sizes of microvessels of the AP, depending on the presence of the pineal gland.

Unique effects of SX in rats and other animals have been long reported from not only our laboratory but also several other laboratories (for review see: KACHI, 1987), although the mechanisms of these effects are unknown. SX has been reported to cause an increase in blood melatonin levels in sea turtles (OWENS and GERN, 1985). However, a clear increase in the mean plasma level of melatonin was not detected in rats, although SX caused an increased variation in the nocturnal plasma melatonin level (KURUSHIMA, 1998). Another possible mechanism of pineal-dependent SX effects in rats is that melatonin (or other pineal hormones) might act on the process(es) driven in cells and systems of the body in response to stressors or other stimuli, such as intracranial surgery, and/or states adapted to these (KACHI, 1987; KACHI et al., 1997; VANECEK, 1998).

In relation to the mechanisms of SX- and PX-induced changes in the luminal sizes of microvessels in the AP, the general rule governing a tight coupling between the neuronal tissue activity and the blood flow in the brain tissue may come to mind (GANONG, 1991; EDVINSSON et al., 1993). However, the situation is different in the circumventricular organs including the AP, which has an abundant supply of capillaries, and experimental data indicate that here the capillary blood stream does not necessarily reflect the local neuronal activity (GROSS, 1992).

Since our present and previous results showed that vasodilatation occurs in PX rats in contrast to SX rats and that the brain water content is higher in SX than in PX rats (KACHI et al., 1997), a hypothesis can be proposed that melatonin causes vasospasm and increased brain water content. This hypothesis is supported by the following experimental evidence: 1) Melatonin receptors exist in the arterial circle of Willis and basilar artery of the brain, and the vertebral and spinal arteries (STANKOV et al., 1993a,b; VISWANATHAN et al., 1993). 2) In young animals, melatonin causes a marked reduction in the blood stream in brain regions supplied with the circle of Willis and basilar artery (CAPSONI et al., 1995). 3) Melatonin receptors also exist in the choroid plexus (STANKOV et al., 1993a,b), which can be stimulated by melatonin (QUAY, 1983) and is the major site for the formation of CSF. Since capillaries in the AP in most mammals are supplied with vessels mainly from the anterior inferior cerebellar arteries — which are branches of the basilar artery, and the posterior spinal arteries, branches of the vertebral arteries (KROIDL, 1968; BRIZZEE and KLARA, 1984; LESLIE, 1986), the decrease in capillary diameters due to SX and the increase due to PX may be caused by the direct action of melatonin on these arteries.

Neural control via NTS neurons is another possible mechanism of vasoconstriction in SX rats and vasodilatation in PX rats, because it is known that: 1) melatonin increases the neural activity of NTS (VITTE et al., 1989), and 2) chemical stimulation of the NTS decreases the blood flow in various brain regions (MAEDA et al., 1990, 1998).

Nuclear size has long been used as a parameter of the functional activity of many kinds of cells (RATHER, 1958). The present results indicated that the nuclei of nerve cells were, at least in part, larger in SX rats than in NO and PX rats. However, no significant differences in the mean nuclear size were recognizable among the three experimental groups. This discrepancy can be interpreted as a direct action of melatonin on partial populations of neurons in the AP, because it has been demonstrated that: 1) melatonin receptors exist in the AP (WEAVER et al., 1989; WILLIAMS, 1989; LAITINEN et al., 1990), 2) the AP neurons consist of heterogeneous populations (LESLIE, 1986; JOHNSON and LOEWY, 1990; FERGUSON, 1992; CAI et al., 1994), and 3) melatonin can exert a stimulatory action on small populations of nerve cells, although melatonin has an inhibitory action on nerve cells in many regions in the brain (for review see: KACHI et al., 1997). Indirect actions of melatonin via neural and/or humoral routes can not be neglected either.

Since it has been reported that the AP has reciprocal neural connections with the NTS and the stimulation of AP modulates neuronal activity of the NTS (SHAPIRO and MISELIS, 1985; CAI et al., 1994), NTS neurons indirectly activated by an increased effect of melatonin on the AP may cause a decrease in the luminal sizes of microvessels in the AP. The possible facilitatory influence of the pineal gland on partial populations of AP neurons may also be involved in the central control mechanism of the sympatho-adrenomedullary system which includes the NTS and adrenalin neurons (FUXE et al., 1988; HÖKFELT et al., 1988; GUYENET, 1990; KACHI et al., 1997). Various autonomic centers including the hypothalamus and cerebral cortex (LOEWY, 1990) may additionally be involved in the pineal hormonal and SX effects.

However, more experimental data are needed to clarify in more detail the mechanisms by which SX and/or melatonin influence the cardiovascular functions, including the cerebral circulation and the sympatho-adrenomedullary system.

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REFERENCES

BLESSING, W. W.: The lower brainstem and bodily homeostasis. Oxford, New York, 1997 (p. 29-99, p. 165-268).

BORISON, H. L.: Area postrema: Chemoreceptor circumventricular organ of the medulla oblongata. Prog. Neurobiol. 32: 351-390 (1989).

BRIZZEE, K. R. and **P. M. KLARA:** The structure of the mammalian area postrema. Fed. Proc. 43: 2944-2948 (1984).

CAI, Y., M. HAY and **V. S. BISHOP:** Stimulation of area postrema by vasopressin and angiotensin II modulates neuronal activity in the nucleus tractus solitarius. Brain Res. 647: 242-248 (1994).

CAPSONI, S., B. M. STANKOV and **F. FRASCHINI:** Reduction of regional cerebral blood flow by melatonin in young rats. Neuroreport 6: 1346-1348 (1995).

DAMPNEY, R. A. L.: Functional organization of central pathways regulating the cardiovascular system. Physiol. Rev. 74: 323-364 (1994).

EDVINSSON, L., E. T. MACKENZIE and **J. MCCULLOCH:** Cerebral blood flow and metabolism. Raven, New York, 1993 (p. 159-180, p. 339-377).

FERGUSON, A. V.: Neurophysiological analysis of mechanisms for subfornical organ and area postrema involvement in autonomic control. Prog. Brain Res. 91: 413-421 (1992).

FUXE, K., A. HARFSTRAND, L. F. AGNATI, M. KALIA, A. NEUMEYER, T. H. SVENSSON, G. VON EULER, L. TERENIUS, P. BERNARDI and **M. GOLDSTEIN:** Physiological and biochemical characteristics of epinephrine/neuropeptide Y-costoring neurons in the medulla oblongata of the rat, with particular reference to vasodepressor functions within the nucleus tractus solitarii. In: (ed. by) J. M. STOLK, D. C. U'PRICHARD and K. FUXE: Epinephrine in the central nervous system. Oxford, New York, 1988 (p. 346-365).

GANONG, W. F.: Review of medical physiology. Appleton & Lange, East Norwalk, 1991 (p. 550-572).

GROSS, P. M.: Circumventricular organ capillaries. Prog. Brain Res. 91: 219-233 (1992).

GUYENET, P. G.: Role of the ventral medulla oblongata in blood pressure regulation. In: (ed. by) A. D. LOEWY and K. M. SPYER: Central regulation of autonomic functions. Oxford, New York, 1990 (p. 145-167).

HÖKFELT, T., G. A. FOSTER, O. JOHANNSON, M. SCHÜLTZBERG, V. HOLETS, G. JU, G. SKAGERBERG, M. PALKOVITS, L. SKIRBOLL, J. M. STOLK, D. C. U'PRICHARD and **M. GOLDSTEIN:** Central phenylethanolamine N-methyltransferase-immunoreactive neurons: Distribution, projections, fine structure, ontogeny, and coexisting peptides. In: (ed. by) J. M. STOLK, D. C. U'PRICHARD and K. FUXE: Epinephrine in the central nervous system. Oxford, New York, 1988 (p. 10-32).

IRIE, T. and **T. KACHI:** A quantitative histological study on neuronal cell nuclei in lateral and ventral horns of the rat spinal cord: Effects of intracranial surgery, pinealectomy and continuous lighting. Hirosaki Med. J. 51: 15-26 (1999).

JOHNSON, A. K. and **A. D. LOEWY:** Circumventricular organs and their role in visceral functions. In: (ed. by) A. D. LOEWY and K. M. SPYER: Central regulation of autonomic functions. Oxford, New York, 1990 (p. 247-267).

KACHI, T.: Pineal actions on the autonomic system. Pineal Res. Rev. 5: 217-263 (1987).

KACHI, T., G. TAKAHASHI, T. SUZUKI, N. KIMURA, S. KAJIHARA, M. KURUSHIMA, T. YAMAUCHI, T. IRIE, H. KUDOU, Y. SAITO, S. HASHIMOTO and **K. HONMA:** Pineal effects on adrenal medulla, area postrema and brain water content in relation to intracranial surgery. Biol. Signals 6: 255-263 (1997).

KACHI, T., G. TAKAHASHI, T. SUZUKI, N. KIMURA, S. KAJIHARA, M. KURUSHIMA, T. YAMAUCHI and **T. IRIE:** Dynamic and versatile structures of adrenal medulla, related to pineal and surgery. In: (ed. by) S. YAGIHASHI, T. KACHI and M. WAKUI: Dynamic cells: Cell biology of the 21st century. Elsevier, Amsterdam, 1998 (p. 47-58).

KROIDL, R.: Die arterielle und venöse Versorgung der Area postrema der Ratte. Z. Zellforsch. 89: 430-452 (1968).

KURUSHIMA, M.: Effects of intracranial surgery on pineal structures and melatonin secretion. Thesis, Hirosaki University, 1998.

LAITINEN, J. T., G. FLÜGGE and **J. M. SAAVEDRA:** Characterization of melatonin receptors in the area postrema: Modulation of affinity with cations and guanine nucleotides. Neuroendocrinology 51: 619-624 (1990).

LESLIE, R. A.: Comparative aspects of the area postrema: Fine-structural considerations help to determine its function. Cell. Mol. Neurobiol. 6: 95-120 (1986).

LOEWY, A. D.: Central autonomic pathways. In: (ed. by) A. D. LOEWY and K. M. SPYER: Central regulation of autonomic functions. Oxford, New York, 1990 (p. 88-103).

MAEDA, M., M. NAKAI, A. J. KRIEGER and **H. N. SAPRU:** Chemical stimulation of the nucleus tractus solitarii decreases cerebral blood flow in anesthetized rats. Brain Res. 520: 255-261 (1990).

MAEDA, M., R. DUELLI, H. SCHRÖCK and **W. KUSCHINSKY:** Autoradiographic determination of local cerebral blood flow and local cerebral glucose utilization of the nucleus tractus solitarii of anesthetized rats. J. Auton. Nerv. Syst. 69: 132-140 (1998).

OWENS, D. W. and W. A. GERN: The pineal gland and melatonin in sea turtles. In: (ed. by) B. LOFTS and W. N. HOLMES: Current trends in comparative endocrinology. Hong Kong University Press, Hong Kong, 1985 (p. 645-648).

QUAY, W. B.: Experimental evidence for pineal participation in homeostasis of brain composition. Prog. Brain Res. 10: 646-653 (1965).

———: Pineal chemistry. Charles C Thomas, Springfield, 1974 (p. 430).

———: Actions of the pineal gland and melatonin on the secretion of cerebrospinal fluid. In: (ed. by) J. AXELROD, F. FRASCHINI and G. P. VELO: The pineal gland and its endocrine role. Plenum, New York, 1983 (p. 349-360).

RATHER, L. J.: The significance of nuclear size in physiological and pathological process. Ergeb. Allg. Pathol. Anat. 38: 127-199 (1958).

SAAVEDRA, J. M.: Brain epinephrine in hypertension and stress. In: (ed. by) J. M. STOLK, D. C. U'PRICHARD and K. FUXE: Epinephrine in the central nervous system. Oxford, New York, 1988 (p. 102-115).

SHAPIRO, R. E. and R. R. MISELIS: The central neural connections of the area postrema of the rat. J. Comp. Neurol. 234: 344-364 (1985).

STANKOV, B., S. CAPSONI, V. LUCINI, J. FAUTECK, S. GATTI, B. GRIDELLI, G. BIELLA, B. COZZI and F. FRASCHINI: Autoradiographic localization of putative melatonin receptors in the brains of two old world primates: *Cercopithecus aethiops* and *Papio ursinus*. Neuroscience 52: 459-468 (1993a).

STANKOV, B., F. FRASCHINI and R. J. REITER: The melatonin receptor: Distribution, biochemistry, and pharmacology. In: (ed. by) H. S. YU and R. J. REITER: Melatonin: biosynthesis, physiological effects, and clinical applications. CRC Press, Boca Raton, 1993b (p. 155-186).

STOLK, J.M., D.C. U'PRICHARD and K. FUXE (eds.): Epinephrine in the central nervous system. Oxford Univ. Press, New York, 1988 (p. 379).

VANECEK, J.: Cellular mechanisms of melatonin action. Physiol. Rev. 78: 687-721 (1998).

VISWANATHAN, M., J.T. LAITINEN and J.M. SAAVEDRA: Vascular melatonin receptors. Biol. Signals 2: 221-227 (1993).

VITTE, P.-A., J. BRUN, P. LESTAGE, B. CLAUSTRA and P. BOBILLIER: The effects of melatonin and pinealectomy upon local cerebral glucose utilization in awake unrestrained rats are restricted to a few specific regions. Brain Res. 489: 271-282 (1989).

VOLLRATH, L.: The pineal organ. In: (ed. by) A. OKSCHE and L. VOLLRATH: Handbuch der mikroskopischen Anatomie des Menschen, VI. Springer, Berlin, 1981 (p. 665).

WEAVER, D. R., S. A. RIVKEES and S. M. REPPERT: Localization and characterization of melatonin receptors in rodent brain by in vitro autoradiography. J. Neurosci. 9: 2581-2590 (1989).

WILLIAMS, L. M.: Melatonin-binding sites in the rat brain and pituitary mapped by in-vitro autoradiography. J. Mol. Endocrinol. 3: 71-75 (1989).

YU, H.-S. and R. J. REITER (eds.): Melatonin: biosynthesis, physiological effects, and clinical applications. CRC Press, Boca Raton, 1993 (p. 550).

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