Time-Dependent Effects of Intrahypothalamic Injection of Vasoactive Intestinal Peptide on Spontaneous Motor Activity in Rats

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Abstract In male Wistar rats, microinjection cannulae were stereotaxically implanted in the ventromedial and paraventricular hypothalamic nuclei. After control recordings of spontaneous motor activity for 4-6 days, vasoactive intestinal peptide (VIP) was injected in a dose of 1.0 μ g and a volume of 2.0 μ l into these nuclei at 1100 or 1900 h, and the activity during the 3-h period following was compared with that on the control days in individual animals. VIP injection produced an increase when injected at 1100 h, and a decrease when injected at 1900 h. Thus, a time-dependent fluctuation in the VIP effect on the hypothalamic neuronal activity was suggested.

Key words: spontaneous motor activity, circadian rhythm, vasoactive intestinal peptide, hypothalamic microinjection, rats.

It has recently been reported that an intracerebroventricular administration of vasoactive intestinal peptide (VIP) shortens the duration of pentobarbital-induced sleep and produces significant hypermotility in the rat, indicating that this peptide plays some role in the manifestation of behavior (ITO *et al.*, 1985).

On the other hand, the hypothalamic nuclei such as the ventromedial and paraventricular hypothalamic nuclei (VM and PV, respectively) were identified as being effective sites for eliciting overeating and increased body weight, when lesioned (HEINBECKER *et al.*, 1944; GROSSMAN, 1975; ROWLEY, 1977; LEIBOWITZ *et al.*, 1981), indicating the involvement of these regions in the control of feeding behavior. Furthermore, it has been indicated that the VM is an important center for the behavioral response to a variety of stimuli. In addition to causing overeating, the lesion causes in rats exaggerated responses to sensory stimuli, i.e., the increase in aggressive behavior, avoidance behavior, and locomotor activity (GROSSMAN, 1972; SEPINWALL, 1969), which have been attributed to increased affectivity (WEISMAN

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and HAMILTON, 1972), a reduction in fear (COLPAERT, 1975), increased sensitivity to pain (GROSSMAN, 1972), and so on. The increased locomotor activity occurred even in a novel environment (SEPINWALL, 1969).

Therefore, in the present experiments, the effects of injection of VIP into the VM and PV on spontaneous motor activity were studied in order to examine whether these regions are the site of action of VIP. Further, whether the effect differed according to the time of injection was also examined since a time-dependent fluctuation was reported previously concerning the effects on the feeding behavior of norepinephrine administered in the lateral hypothalamus (MARGULES *et al.*, 1972).

MATERIALS AND METHODS

Animals. Male Wistar rats, weighing approximately 300 g, were housed at a constant temperature of 25°C under a 14-h/10-h light-dark cycle (lights on 0500–1900 h), with food and water available *ad libitum*.

Surgery. A total of 29 animals were anesthetized with sodium pentobarbital, and then were implanted unilaterally with the guide cannulae made of a stainless steel tube with a 0.65 mm outer diameter into the hypothalamus, according to the atlas of ALBE-FESSARD *et al.* (1966). The coordinates were as follows: for the PV, $A=6.6$, $L=0.5$, $V=3.3$; for the VM, $A=6.6$, $L=0.5$, $V=2.0$. The cannulae were fixed to the skull with dental cement and stainless steel screws. The inner cannula with a 0.30 mm outer diameter was arranged so as to protrude approximately 0.5 mm beyond the tip of the guide cannula.

Peptide injection. VIP was injected into the hypothalamic nuclei in a dose of 1.0 μg (about 0.3 nmol) and a volume of 2.0 μl using a 10 μl microsyringe attached to the inner cannula with Silastic tubing at the speed 1.0 $\mu\text{l}/\text{min}$. Synthetic VIP was purchased from the Peptide Institute, Minoo, Japan. It was initially dissolved in 0.001 M HCl and diluted with saline to obtain the final concentration. The dose 1.0 μg was reported to be minimally effective to produce hypermotility when injected intracerebroventricularly (ITO *et al.*, 1985).

Spontaneous motor activity. Two weeks after the implantation of the guide cannula, each animal in the individual cage was transferred to an experimental chamber, in which the same light-dark cycle was maintained as before. Spontaneous motor activity was measured with an Automex (Columbus Instruments), an instrument that displays magnetic field counts corresponding to increased activity of the rat in the cage, and the counts were recorded every 30 min. After recording for 4–6 days during which no drugs were injected (therefore, designated as control days), each animal was tested with an intrahypothalamic injection of saline (2.0 μl) or VIP. The injection was performed at 1100 h for the light-cycle test or 1900 h for the dark-cycle test, and the recording was continued for another 8 h, 16 h, or more. Although it was a rule that every animal received both the light- and dark-cycle tests for saline, all the animals did not necessarily receive both tests for VIP. Further, the

experiment was discontinued if an animal did not show an apparent circadian rhythm in the activity for some reason on control days. This occurred in 4 rats.

Histology and statistical analysis. After the recording experiment was finished, all the animals were anesthetized with ether and perfused with 10% buffered formalin. The site of injection was microscopically identified in 40 μ m sections stained with cresyl violet.

The evaluation of the effect of peptide on the motor activity was performed for each injection by comparing the total activity count during the 3-h period following the injection, i.e., 1100–1400 h or 1900–2200 h, with the mean of the total activity count during the corresponding 3-h period on the control days in the same animal. That is, if the activity count during the 3-h period after saline or peptide injection was increased or decreased beyond 2 S.D. of the mean activity count during the corresponding 3-h period on the 4–6 control days, the effect of saline or peptide was evaluated as facilitation or inhibition, respectively. Data from 3 animals which showed a facilitation in response to saline as well as to VIP during light cycle were eliminated from further analysis, since such response to VIP was assumed to be nonspecific. Additional evaluation was performed similarly for the total activity count during the 10-h period in the dark cycle.

RESULTS

A total of 33 injections of VIP, which were performed in 22 rats, were evaluated for the effect on motor activity as described before. Each evaluation is shown in Fig. 1, with respect to the site of injection. Further, these results are summarized in Table 1, with respect to each of the hypothalamic nuclei, i.e., the VM and PV, and other regions, i.e., anterior hypothalamic area (AHA) and dorsomedial

Table 1. Effect of VIP injection in the hypothalamic nuclei on spontaneous motor activity.

Sites	Time of injection							
	Light cycle (1100 h)				Dark cycle (1900 h)			
	No. of injections	Facilitation	No change	Inhibition	No. of injections	Facilitation	No change	Inhibition
PV	5	4*	1	0	4	0	1	3
VM	11	8	3	0	6	1	2	3
AHA	3	0	3	0	0	0	0	0
DM	3	0	1	2	1	0	0	1

* Indicates the number of injections that produced facilitation, no change, or inhibition.

Fig. 1. Schematic illustration showing the site of the tip of the cannula in the brain. According to the effect of peptide on the spontaneous motor activity, the site is shown by for facilitation (▲), for inhibition (▼), or for no change (●). The illustrations are modified from the atlas of ALBE-FESSARD *et al.* (1966). Abbreviations: ar, arcuate nucleus; CC, corpus callosum; C hip, corpus hippocampi; DM, dorsomedial hypothalamic nucleus; Fx, fornix; PV, paraventricular hypothalamic nucleus; VM, ventromedial hypothalamic nucleus.

hypothalamic nucleus (DM), in which several cannulae were located unexpectedly.

Twelve (75%) of 16 injections of VIP in the VM and PV performed at 1100 h produced an increase in motor activity in the 3-h period afterward. The mean ($\pm$ S.E.) of the increase rate produced by the 12 injections was $308 \pm 53\%$, when compared to the mean activity count during the corresponding period on control days. However, the injection at 1900 h produced a reduction to $40 \pm 12\%$ in 6 (60%) of 10 rats injected. Only one injection, which was performed rather in a more caudal part of the VM than in others, caused an increase. Such time-dependent difference in the VIP effect was significant as analyzed by chi-square test for both the VM and PV (chi-square (2)=14.857, $p < 0.001$), and also for each of the VM (chi-square (2)=7.853, $p < 0.05$) and the PV (chi-square (2)=6.975, $p < 0.05$). Three

Fig. 2. Representative profiles of spontaneous motor activity recorded in individual animals. Facilitatory effects of VIP injected in the VM at 1100 h on the activity counts in the 3-h period afterward (shaded area) are shown. Numbers in the parentheses indicate the rat No. Black sections in the horizontal bar indicate the dark cycle (1900–0500 h) and arrows indicate the injection.

injections in the AHA performed at 1100 h did not cause a significant effect, while 3 of 4 injections in the DM produced an inhibition regardless of the time they were performed.

In Figs. 2 and 3, representative profiles of spontaneous motor activity recorded in individual animals are shown. In rat Nos. 144 and 186 (Fig. 2), VIP injection into the VM at 1100 h produced a significant increase in the activity during the period 1100–1400 h (in rat No. 144, 843 counts in the period 1100–1400 h after saline injection whereas 1,513 counts after VIP; in rat No. 186, 666 counts after saline and

Fig. 3. Representative profiles of spontaneous motor activity recorded in individual animals. Inhibitory effects of VIP injected in the VM (upper) and PV (lower) at 1900 h are shown. For further details, see Fig. 2.

1,939 after VIP). In contrast, significantly inhibitory effects of VIP were caused when injected into the VM at 1900 h (Fig. 3). In rat No. 166, VIP injection at 1100 h produced a small but significant increase in the activity (the activity count after saline was 90, although not shown in the figure, and that after VIP was 218), whereas the injection at 1900 h produced a significant decrease in the period 1900–2200 h (1,785 counts after saline, but 186 counts after VIP) and also in the period 1900–0500 h. The injection at 1900 h into the PV in rat No. 187 also produced a decrease, in either the period 1900–2200 h (3,437 counts after saline, but 1,692 after VIP, and in this case, VIP effects during the light cycle was not

significant, although the data after saline is not shown in the figure) or the period 1900–0500 h.

DISCUSSION

The hypermotility, which was produced by the intracerebroventricular injection of VIP in the rat during the light cycle (ITO *et al.*, 1985), was shown to be reproduced by the VIP injection in the VM and PV in the present study. It was then assumed that these hypothalamic nuclei somehow stimulate the motor activity in response to VIP in the light cycle. As mentioned before, the hypothalamic nuclei which we tested in the present study were almost those which have been shown to be involved in the control of feeding behavior and behavioral response to a variety of stimuli. Therefore, it is very probable that the effect of VIP on spontaneous motor activity will involve an effect on these behaviors. Further, the present study showed that the effect of VIP on the motor activity was reversed and therefore was inhibitory, when the injection was performed in the dark cycle. Then, the injection of VIP in the medial hypothalamus seemed to cause opposite effects on motor activity that depended on the time of day the peptide was injected.

There is increasing evidence to support VIP as a neurotransmitter or neuromodulator in the brain (ROSSELIN *et al.*, 1982). In the hypothalamus, VIP has been shown to stimulate adenylate cyclase activity (QUIK *et al.*, 1978; BORGHI *et al.*, 1979) and alter neuronal electrical activity (HASKINS *et al.*, 1982; AKEMA *et al.*, 1986). When injected in the third ventricle, VIP affects the anterior pituitary hormone secretion, probably acting at the hypothalamus (VIJAYAN *et al.*, 1979; ALEXANDER *et al.*, 1985). Together with the finding that VIP has a marked regional distribution with considerably high levels in the hypothalamic nuclei (see review by PALKOVITS, 1985), it is probable that VIP administered in the hypothalamus affected the neuronal activity acting as a neurotransmitter. However, a possibility cannot be denied that it has altered local metabolism in the hypothalamus by causing vasodilation of cerebral blood vessels (DUCKLES and SAID, 1982) and enhancing glucose utilization (MAGISTRETTI *et al.*, 1981).

Although it has not been determined how injected VIP caused time-dependent effects on the neuronal activity, we propose two possibilities: first, injected VIP inhibited the neuronal activity of the PV and VM in the light cycle but facilitated it in the dark cycle, and second, injected VIP potentiated the response of PV and VM neurons to endogenous norepinephrine which was inhibition in the light cycle but facilitation in the dark cycle.

Taking the finding into consideration that rats with destruction of the medial hypothalamus overeat both in the light and dark cycle, not displaying a diurnal variation (AHLSSKOG *et al.*, 1975), whereas normal rats eat more food in the dark cycle, indicating that the medial hypothalamus is acting to inhibit feeding even in the light cycle, it was possible that injected VIP inhibited the neuronal activity in the medial hypothalamus in the light cycle whereas it facilitated it during the dark cycle.

Such a time-dependent difference in the effect of VIP suggests an implication of influence of circadian rhythm mechanism whose center locates in the suprachiasmatic nucleus (MOORE, 1978). Namely, the suprachiasmatic nucleus contains a very dense concentration of VIP-reactive perikarya and gives fibers to some of the hypothalamic nuclei including the PV and VM (SIMS *et al.*, 1980) and further, this region in the hypothalamus has been shown to provide the neuronal activity of many other regions with circadian rhythmicity which is generally low in the light cycle and high in the dark cycle (INOUE and KAWAMURA, 1979). Taken together, there seems to be a possibility that an influence from the suprachiasmatic nucleus which is mediated by VIP fibers causes an inhibition in the light cycle and a facilitation in the dark cycle in the hypothalamic neuronal activity. Then, it was likely that the addition of exogenous VIP would have potentiated such endogenous VIP effects on these brain regions.

The second possibility is based on the assumption that norepinephrine, acting at the hypothalamic nucleus, causes time-dependent effects on feeding. When administered directly in the PV or VM during the light cycle, it induced feeding responses in satiated rats (LEIBOWITZ, 1978; MATTHEWS *et al.*, 1978), and when administered in the lateral hypothalamus, it inhibited feeding during the light cycle and facilitated during the dark cycle (MARGULES *et al.*, 1972) in rats. Taking the finding into consideration that destruction of noradrenergic bundles with 6-hydroxydopamine facilitated feeding only in the dark cycle (AHLSSKOG *et al.*, 1975), it seems that the noradrenergic transmission exerts, as a whole, an influence on the hypothalamic nuclei to prevent overeating in the dark cycle, whereas it exerts some facilitatory influence in the light cycle. On the VM and PV, the norepinephrine effect is assumed to be inhibitory in the light cycle but facilitatory in the dark cycle.

An existence of the synergism between VIP and norepinephrine has been suggested in the cerebral cortex, in which VIP and norepinephrine fiber systems have the same targets, the pyramidal cells (see review by BLOOM *et al.*, 1985). There, VIP alone causes either inhibitions, excitations, and no changes of neuronal discharges, whereas the effects on norepinephrine are predominantly inhibitory. However, if VIP was applied during administration of subthreshold norepinephrine, the norepinephrine effects were pronounced inhibitions of neuronal discharge regardless of the effect of VIP prior to norepinephrine; even in cases where VIP alone gave excitatory effects, concurrent subthreshold norepinephrine treatment reversed the VIP effect from excitation to inhibition, implying, in turn, that VIP acts to augment the action of norepinephrine on neuronal discharges in the cortex. Further, it was found that VIP and norepinephrine could act synergistically to increase adenylate cyclase in the cortical slices (MAGISTRETTI and SCHORDERET, 1985). It is thus possible that the exogenous VIP administered will potentiate the action of endogenous norepinephrine on the neuronal activity of the PV and VM, which will be either facilitatory or inhibitory in the hypothalamus depending on the time of day, and thus will reveal its time-dependent effects, as far as feeding is concerned.

It is interesting to consider that the effects of VIP will probably change as a

function of norepinephrine activity at the target neurons. It is possible that the first mechanism we raised for the reversed effects of VIP due to the time of day virtually involves this VIP-norepinephrine interaction at the levels of hypothalamic neurons. However, studies are needed to ascertain the mechanism for VIP effects on motor activity.

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