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RAPID COMMUNICATIONS

INVOLVEMENT OF HYPOTHALAMIC VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IN PROLACTIN SECRETION INDUCED BY SEROTONIN IN RATS[1]

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Abstract. To study the possible involvement of hypothalamic vasoactive intestinal polypeptide (VIP) in regulating the secretion of prolactin (PRL), the effect of anti-VIP rabbit serum on serotonin (5-HT)-induced PRL release was examined in urethane-anesthetized male rats. Anti-VIP serum (AVS) or normal rabbit serum (NRS) was infused into a single hypophysial portal vessel of the rat for 40 min at a rate of 2 μl/min with the aid of a fine glass cannula and 5-HT was injected into a lateral ventricle 10 min after the start of the infusion. Intraventricular injection of 5-HT (10 μg/rat) caused an increase in plasma PRL levels in control animals infused with NRS and 5-HT-induced PRL release was blunted in animals infused with AVS (mean±SE peak plasma PRL: 118.9±19.8 ng/ml *vs* 54.7±16.2 ng/ml, $p<0.05$). These findings suggest that the secretion of PRL induced by 5-HT is mediated, at least in part, by hypothalamic VIP release into the hypophysial portal blood in the rat.

Vasoactive intestinal polypeptide (VIP) is widely distributed throughout the central nervous system (1,2). The presence of VIP in hypothalamic nerve endings (3) and in hypophysial portal blood (4,5) suggests that hypothalamic VIP is involved in regulating the secretion of pituitary hormones. VIP stimulates prolactin (PRL) secretion in the rat by acting, at least in part, at the pituitary level (6-9). However, the physiological role of endogenous VIP in regulating PRL secretion remains to be determined. We have previously reported that brain serotonergic mechanisms play a stimulating role in regulating hypothalamic VIP release (10,11) and pituitary PRL secretion (12,13). In the present study, we report the possible involvement of hypothalamic VIP in PRL secretion induced by serotonin (5-HT) using a passive immunization method.

Materials and Methods. Wistar strain male rats weighing 350-450 g (Japan Animal Co., Osaka) were maintained in a temperature controlled room (23±1°C) on a 12 h dark, 12 h light schedule (lights on 0600-1800). Laboratory chow (Oriental Yeast Co., Tokyo) and tap water were given *ad libitum*.

After overnight fasting, animals were anesthetized with urethane (150 mg/100 g body wt., ip) and the pituitary stalk was exposed by the parapharyngeal approach as described by Porter *et al* (14). A fine glass cannula was inserted into a main portal vessel and anti-VIP rabbit serum (AVS) or normal rabbit serum (NRS), diluted with physiological saline (1:10), was infused at a rate of 2 μl/min for 40 min by means of an infusion pump (Harvard Apparatus, MA)(15). Serotonin creatinine sulfate was dissolved in physiological saline and injected into a

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lateral ventricle in a volume of 10 µl/rat via an implanted cannula (PE10) 10 min after the start of infusion.

Blood samples of 0.4 ml were withdrawn from the jugular vein at 10-20 min intervals during the experiments as previously described (6). Plasma samples were promptly separated and kept at -20 °C until assayed. PRL concentrations in the plasma were measured by specific radioimmunoassay using a kit supplied from the NIADDK (6,13). NIADDK-rat prolactin RP-1 was used for the standard. Student's t test was used for the statistical evaluation.

AVS(R502) was generated in a rabbit against the mixture of synthetic VIP and polyvinyl pyrrolidone (16). The antigenic determinant sites were mid portion and C-terminal residues of the VIP molecule, showing no cross reactivity with porcine intestinal peptide histidine isoleucine (PHI), secretin, glucagon, human pancreatic growth hormone releasing factor (hpGRF) and rat GRF. Its avidity and binding capacity were 1.3×10^{11} 1/mol and 0.9 nmol/ml, respectively.

Results. As shown in Fig. 1, intraventricular administration of 5-HT (10 µg/rat) caused a significant increase in plasma PRL levels in control animals which were infused with NRS via a hypophysial portal vessel. Peak values of plasma PRL were obtained 20 min after 5-HT injection. Plasma PRL levels raised by 5-HT were significantly suppressed in animals infused with AVS (mean±SE peak plasma PRL: NRS 118.9±19.8 ng/ml vs AVS 54.7±16.2 ng/ml, $p<0.05$). Integrated PRL release induced by 5-HT during the infusion period was also suppressed by AVS (NRS 228±40 ng/ml vs AVS 82±40 ng/ml, $p<0.05$). Basal levels of plasma PRL were not affected by the infusion of AVS or NRS into a hypophysial portal vessel.

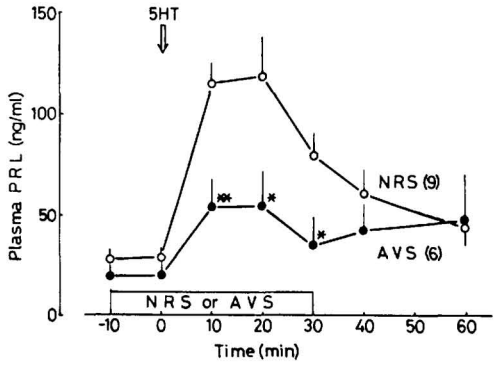

Fig. 1. Effect of the infusion of anti-VIP rabbit serum (AVS) into a single hypophysial portal vessel on prolactin release induced by 5-HT in the rat. AVS or normal rabbit serum (NRS) as a control was infused at a rate of 2 µl/min for 40 min. 5-HT (10 µg/rat) was injected intraventricularly 10 min after starting the infusion. All values are the mean±SE of 6 and 9 rats as indicated in parentheses. Statistical differences are shown by asterisks (* $p<0.05$, ** $P<0.01$ vs NRS).

Discussion. It is well known that 5-HT stimulates the secretion of PRL, acting through the central nervous system (12, 17-19). However, the mechanism by which 5-HT influences PRL release remains to be elucidated. We have previously reported that VIP release from the hypothalamus is stimulated by 5-HT in vivo and in vitro (10,11). VIP is currently viewed as a putative PRL releasing factor (PRF), since it is present in the hypothalamus (1,2) and secreted into the hypophysial portal blood (4,5) in high concentration sufficient to stimulate PRL release from the pituitary lactotroph in vitro (7-9). It is postulated, therefore, that hypothalamic VIP may be involved in 5-HT-induced PRL secretion.

In the present study, we showed that plasma PRL response to 5-HT was significantly blunted by anti-VIP serum which was locally infused into a hypophysial portal vessel to neutralize VIP activity in hypophysial portal plasma and in the venous plexus of the pituitary.

These findings strongly suggest that hypothalamic VIP is involved, at least in part, in the secretion of PRL induced by 5-HT in the rat.

1. Loren I, Emson PC, Fahrenkrug J, Björklund A, Aumets J, Håkanson R, Sundler F, Distribution of vasoactive intestinal polypeptide in the rat and mouse brain. Neuroscience 4:1953-1976, 1979.
2. Sims KB, Hoffman DL, Said SI, Zimmerman EA, Vasoactive intestinal polypeptide (VIP) in mouse and rat brain: an immunocytochemical study. Brain Res 186:165-183, 1980.
3. Giachetti A, Said SI, Reynolds RC, Koniges FC, Vasoactive intestinal

polypeptide in brain: localization in and release from isolated nerve terminals. Proc Natl Acad Sci USA 74: 3424-3428, 1977.
4. Said SI, Porter JC, Vasoactive intestinal polypeptide: release into hypophyseal portal blood. Life Sci 24:227-230, 1979.
5. Shimatsu A, Kato Y, Matsushita N, Katakami H, Yanaihara N, Imura H, Immunoreactive vasoactive intestinal polypeptide in rat hypophysial portal blood. Endocrinology 108:395-398, 1981.
6. Kato, Y., Iwasaki Y, Iwasaki J, Abe H, Yanaihara N, Imura H, Prolactin release by vasoactive intestinal polypeptide in rats. Endocrinology 103:554-558, 1978.
7. Ruberg M, Rostejn WH, Aranchibia S, Besson J, Enjalbert A, Stimulation of prolactin release by vasoactive intestinal polypeptide (VIP). Eur J Pharmacol 51:319-320, 1978.
8. Shaar CJ, Clemens JA, Dininger NB, Effect of vasoactive intestinal polypeptide on prolactin secretion release in vitro. Life Sci 25:2071-2074, 1979.
9. Matsushita N, Kato Y, Shimatsu A, Katakami H, Yanaihara N, Imura H, Effects of VIP, TRH, GABA and dopamine on prolactin release from superfused rat anterior pituitary cells. Life Sci 32:1263-1269, 1983.
10. Shimatsu A, Kato Y, Matsushita N, Katakami H, Yanaihara N, Imura H, Stimulation by serotonin of vasoactive intestinal polypeptide release into rat hypophysial portal blood. Endocrinology 111:338-340, 1982.
11. Shimatsu A, Kato Y, Matsushita N, Katakami H, Ohta H, Yanaihara N, Imura H, Serotonin stimulates vasoactive intestinal polypeptide release from rat hypothalamus in vitro. Brain Res 264:148-151, 1983.
12. Ohgo S, Kato Y, Chihara K, Imura H, Maeda K, Effect of hypothalamic surgery on prolactin release induced by 5-hydroxytryptophan (5-HTP) in rats. Endocrinol Jpn 23:485-491.
13. Matsushita N, Kato Y, Katakami H, Shimatsu A, Imura H, Inhibition of naloxone of prolactin release induced by l-5-hydroxytryptophan in rats. Proc Soc Exp Biol Med 168:282-285, 1981.
14. Porter JC, Mical RS, Kamberi IA, Grazia YR, A procedure for the cannulation of a pituitary stalk portal vessel and perfusion of the pars distalis in the rat. Endocrinology 87:197-201, 1970.
15. Abe H, Kato Y, Chihara K, Iwasaki Y, Imura H, Effects of drugs infused into rat hypophysial portal vessel on prolactin and growth hormone release. Proc Soc Exp Biol Med 165: 248-252, 1980.
16. Yanaihara Y, Sakagami M, Sato H, Yamamoto K, Hashimoto T, Yanaihara C, Ito Z, Yamaguchi K, Abe K, Immunological aspects of scretin, substance P and VIP. Gastroenterology 72:803-810, 1977.
17. Kamberi IA, Mical RS, Porter JC, Effects of melatonin and serotonin on the release of FSH and prolactin. Endocrinology 88:1288-1293, 1971.
18. Lu KH, Meites J, Effects of serotonin precursors and melatonin on serum prolactin release in rats. Endocrinology 93:152-155, 1973.
19. Clemens JA, Doush ME, Fuller RW, Evidence that serotonin neurons stimulates secretion of prolactin releasing factor. Life Sci 22:2209-2214, 1978.

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