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RAPID COMMUNICATIONS

INVOLVEMENT OF PEPTIDE HISTIDINE ISOLEUCINE (PHI) IN PROLACTIN SECRETION INDUCED BY SEROTONIN IN RATS[1]

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Abstract. The possible role of hypothalamic peptide histidine isoleucine (PHI) in prolactin (PRL) secretion induced by serotoninergic mechanisms was investigated in male rats using a passive immunization technique. Intracerebroventricular injection of serotonin (5HT, 10 μg/rat) raised plasma PRL levels both in urethane-anesthetized rats and in conscious rats pretreated with normal rabbit serum (0.5 ml/rat, iv, 30 min before). Plasma PRL responses to 5HT were blunted in these animals when they were pretreated with rabbit antiserum specific for PHI (0.5 ml/rat, iv, 30 min before)(mean±SE peak plasma PRL: anesthetized rats 271.3±38.3 ng/ml vs 150.0±12.6 ng/ml, $p<0.01$, conscious rats 54.3±6.8 ng/ml vs 30.7±4.1 ng/ml, $p<0.025$). These results suggest that hypothalamic PHI is involved, at least in part, in PRL secretion induced by central serotoninergic stimulation in the rat.

Peptide histidine isoleucine (PHI) is a 27 amino acid peptide originally isolated from the porcine intestine by Tatemoto and Mutt (1). Subsequent studies have revealed that PHI exists in the brain including the hypothalamus of several mammalian species (2-5).

PHI has not only a remarkable sequence homology with vasoactive intestinal polypeptide (VIP) but also a common precursor molecule (6,7). We have previously reported that immunoreactive PHI and VIP are highly concentrated in rat hypophysial portal blood (8).

Stimulating effects of both PHI and VIP on pituitary prolactin (PRL) secretion were demonstrated in vivo and in vitro in the rat (9-14). We have recently reported that hypothalamic VIP is involved in rat PRL secretion induced by serotonin (5HT)(15-18). In the present study, we examined the possible involvement of endogenous hypothalamic PHI in PRL secretion induced by 5HT using a passive immunization method in the rat.

Materials and Methods. Wistar strain male rats weighing 250-350 g (Japan Animal Co., Osaka) were used throughout the experiments. They 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.

Experiments were performed with either urethane-anesthetized rats or conscious animals. In animals anesthetized with urethane (150 mg/100 g body wt., ip), test substances were injected into a lateral ventricle (icv) in a volume of 10 μl/rat after overnight fasting as previously described (9). In conscious rats, in which intraatrial and intraventricular catheters were chronically implanted as previously described (19), test substances were injected through the intraventricular catheter after the fasting period of 2 h. Anti-PHI rabbit

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serum (APRS) or normal rabbit serum (NRS) was injected into the exposed jugular vein or through the intraatrial catheter in a volume of 0.5 ml/rat 30 min before the injection of test substances.

Blood samples of 0.6 ml were withdrawn from the jugular vein or through the intraatrial catheter 30 min and immediately before, 10, 20 and 40 min after the injection of test substances. Plasma was promptly separated and kept at -20°C until assayed. Blood cells suspended in saline solution were supplemented after sampling in conscious rats.

Plasma PRL concentrations were measured by specific radioimmunoassay using a kit supplied from the NIADDK as previously described (17). NIADDK-rat prolactin RP-1 was used as the standard. The PHI binding capacity of APRS-treated rat plasma was determined in vitro by calculating the bound percent of ^{125}I-PHI (approximately 60 pg) added to 0.1 ml of rat plasma diluted to 1:50 according to the radioimmunoassay for PHI (5).

APRS (KPR-1) was generated in a rabbit against synthetic porcine PHI(1-27) (Peninsula Lab., Belmont) conjugated to bovine thyroglobulin by glutaraldehyde method. Kd and Bmax of the antiserum were 6.1×10^{-11} M, 8.4 nmol/ml, respectively. The APRS was specific for C-terminal residues of porcine PHI, showing no cross reactivity with porcine PHI(1-15), VIP, secretin, glucagon, TRH, human growth hormone releasing factor (GRF) and rat GRF (20). The extract of rat hypothalamus showed a parallel displacement of ^{125}I-PHI(1-27) binding to the antiserum with porcine synthetic PHI(1-27) and PHI(14-27).

Analysis of variance and Student's t-test were used for statistical evaluation.

Results. Plasma PRL levels were increased by intracerebroventricular (icv) injection of 5HT (10 μg/rat) in urethane-anesthetized rats pretreated with NRS (0.5 ml/rat, iv). Peak plasma PRL values were obtained at 10 min after 5HT injection in these animals. When APRS (0.5 ml/rat) was injected iv in the animals, PRL release induced by 5HT was significantly suppressed (mean±SE peak plasma PRL: NRS 271.5±38.3 ng/ml vs 156.0±12.6 ng/ml, $p<0.01$) (Fig. 1).

Pretreatment with APRS (0.5 ml/rat, iv) also blunted plasma PRL responses to 5HT (10 μg/rat, icv) in conscious rats compared with that of NRS (NRS 54.3±6.8 ng/ml vs 30.7±4.1 ng/ml, $p<0.025$) (Fig. 2).

Diluted rat plasma obtained 40 min after the injection of 5HT was capable of binding of 37% of ^{125}I-PHI added in vitro.

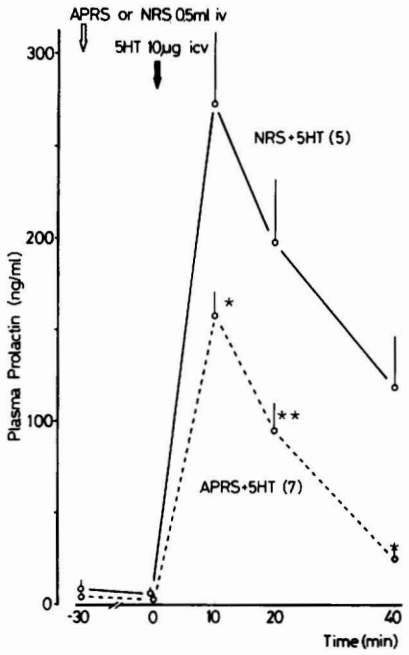

Fig. 1. Effect of intravenous (iv) injection of anti-PHI rabbit serum (APRS) on prolactin release induced by serotonin (5HT) in urethane-anesthetized male rats. APRS or normal rabbit serum (NRS) was injected 30 min before 5HT injection (10 μg/rat, icv). All values are the mean±SE of 5-7 rats indicated in parentheses. * $p<0.01$, ** $p<0.025$ vs NRS.

Discussion. It is well known that 5HT stimulates the secretion of PRL by acting through the central nervous system (21-23). We have previously reported that hypothalamic VIP is involved, at least in part, in the secretion of PRL induced by 5HT in the rat, since 5HT stimulated VIP release from the hypothalamus (16,17) and immunoneutralization of endogenous VIP with anti-VIP serum blunted PRL release induced by 5HT in the rat (18-20).

In the present study, we found that passive immunization with anti-PHI rabbit serum suppressed PRL release induced by 5HT in both urethane-anesthetized rats and conscious animals.

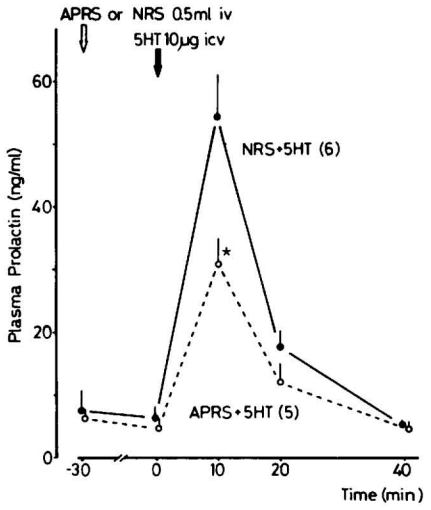

Fig. 2. Effect of anti-PHI rabbit serum (APRS) on prolactin release induced by serotonin (5HT) in conscious rats. APRS or normal rabbit serum (NRS) was injected 30 min before 5HT injection. All values are the mean±SE of 5-6 rats indicated in parentheses. * $p<0.025$ vs NRS.

The antiserum which we used were made by immunizing a rabbit with synthetic porcine PHI(1-27) and completely cross-react with porcine PHI(14-27) but not with PHI(1-15), suggesting that the antigenic determinant sites were C-terminal residues of the PHI molecule (20). Although rat PHI has not been isolated, the amino acid sequence of rat PHI recently identified from the nucleotide sequence for the precursor molecule (7) differs by 2 amino acids from that of porcine PHI. Phe^{10} and Leu^{17} of porcine PHI are replaced by Tyr^{10} and Ile^{17} in rat PHI, respectively. The C-terminal residues of PHI of both species are identical. Rat hypothalamic extract showed a parallel displacement curve with porcine PHI(14-27)(5). It is suggested, therefore, that the antiserum possibly neutralize endogenous rat PHI in the present study.

Partial suppression by the antiserum of PHI release induced by 5HT may indicate either incomplete neutralization of endogenous PHI or a possible involvement of other mechanisms in 5HT-induced PRL secretion in these animals. The interaction between PHI and VIP remains to be further investigated.

It is concluded that hypothalamic PHI is involved, at least in part, in the secretion of PRL induced by serotoninergic stimuli in the rat.

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