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BRIEF COMMUNICATION

Effects of Various Reproductive Hormones on the Penetration of LHRH Across the Blood-Brain Barrier

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BARRERA, C. M., W. A. BANKS, M. B. FASOLD AND A. J. KASTIN. *Effects of various reproductive hormones on the penetration of LHRH across the blood-brain barrier.* PHARMACOL BIOCHEM BEHAV **41**(1) 255–257, 1992.—A previous study has shown bidirectional saturable transport of LHRH across the blood-brain barrier. Here, the effects of the steroids progesterone and β-estradiol and the pituitary glycoproteins luteinizing hormone (LH) and follicle stimulating hormone (FSH) on the bidirectional transport rate were determined. No statistically significant difference in brain to blood transport of ^{125}I-LHRH was found in mice given ICV progesterone (1 and 100 pmol/mouse), β-estradiol (1 and 100 pmol/mouse), FSH (10 and 1000 pmol/mouse) or LH (100 and 1000 pmol/mouse). Blood to brain transport of ^{125}I-LHRH, tested in rats with a carotid artery perfusion method, was not affected by inclusion of progesterone (100 nmol/ml), β-estradiol (100 nmol/ml), LH (2 and 10 nmol/ml), or FSH (10 nmol/ml) in the perfusate. We conclude, therefore, that unlike its release from the hypothalamus, the exchange of LHRH between the CNS and blood is unlikely to be influenced by reproductive hormones.

Luteinizing hormone Follicle stimulating hormone Estrogen Progesterone Testosterone
Luteinizing hormone releasing hormone Blood-brain barrier

LUTEINIZING hormone releasing hormone (LHRH) is a decapeptide released at the median eminence that affects sexual behavior by releasing luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary. Its release, in turn, is affected by the sex steroids. However, exogenous LHRH has been shown to exert effects on sexual behavior independent of the pituitary (6, 9, 11), presumably by gaining direct access to the brain. Recently, LHRH has been shown to be transported in both the brain to blood and blood to brain directions by a saturable mechanism (5), which may explain how LHRH can exert its central actions. The purpose of this study was to determine whether the principal reproductive hormones known to be released by or influencing the release of LHRH from the hypothalamus also affect penetration of LHRH across the blood-brain barrier (BBB). Accordingly, the pituitary hormones LH and FSH and the steroid hormones β-estradiol and progesterone were tested for their effects on the bidirectional saturable transport of LHRH across the BBB.

METHOD

Materials

Male ICR mice, 19–21 g, were purchased from Blue Spruce Farms (Altamont, NY) and male Sprague-Dawley rats, 300–350 g, were purchased from Harlan Sprague-Dawley Laboratories (Indianapolis, IN). They were used no earlier than 2–3 days after arrival. Food (Purina Lab Rodent Chow) and water were freely available and the light:dark cycle was set at 12 h:12 h from 6 a.m. to 6 p.m. Buffer for intracarotid perfusions was prepared as previously described (4). All intracerebroventricular (ICV) injections were given in 1% bovine serum albumin (BSA) to prevent ^{125}I-LHRH from sticking to the syringe. Progesterone and β-estradiol (Sigma Chemical Co., St. Louis, MO) were initially solubilized in 100% ethanol for a final concentration of 10% ethanol in buffer. Ovine LH and FSH were obtained from the National Institute of Diabetes and Digestive and Kidney Disease (NIDDK) and the National Hormone and Pituitary Program (NHPP), University of Maryland School of Medicine.

Iodination

LHRH (Bachem, Philadelphia, PA) was iodinated at the tyrosine position with Na^{125}I (Amersham, Arlington Heights, IL) by the addition of 10 μl of a 10 μM solution of chloramine T for 60 s. The reaction was stopped with 100 μl of 25% BSA (Sigma Chemical Co., St. Louis, MO). The iodinated LHRH

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was then purified by high performance liquid chromatography (HPLC) (Pump model No. 2350; Gradient programmer model No. 2360; ISCO, Lincoln, NE), and kept refrigerated at 4°C until use.

Brain to Blood Studies

Intracerebroventricular injections of ^{125}I-LHRH. Mice were fully anesthetized with IP urethane, 40% in 0.45% NaCl. ^{125}I-LHRH was injected by the method of Noble et al. (8), modified to accurately quantify the movement of substances out of the CNS (1–3). Briefly, 1.0 μl of lactated Ringer's solution with 1% BSA containing 25,000 cpm of ^{125}I-LHRH was injected into the lateral ventricle of the mouse with a 1.0 μl Hamilton syringe (Hamilton Co., Reno, NV). Mice were decapitated 10 min after injection and the brains immediately removed for counting in a gamma counter. The results were expressed with the standard error as percent of the baseline transport ($\%T_B$):

$$\%T_B = 100\ (A-E)/(A-C)$$

where A is cpm available for transport (14,987) as previously determined (5), C is the cpm in control mice and E is the cpm in experimental mice.

In the first ICV experiment, mice received a 1 μl injection containing 25,000 cpm/mouse of ^{125}I-LHRH alone or with unlabeled LH (100 or 1000 pmol/mouse) or FSH (10 or 1000 pmol/mouse). In the second ICV experiment, the unlabeled material was either β-estradiol (1 or 100 pmol/mouse) or progesterone (1 or 100 pmol/mouse).

Blood to Brain Studies

Intracarotid perfusion of ^{125}I-LHRH. After being properly anesthetized with 300–400 μl pentobarbital sodium (65 mg/ml, IP), the rats were placed supine. The cervical region of each rat was surgically exposed and the right and left common carotid arteries were isolated with surgical thread and readied for occlusion (13). The right common carotid was then clamped at the base of the neck and cannulated distally with PE-50 tubing (Intramedics, NJ). The left common carotid artery was occluded within 10 s after the start of the perfusion. The perfusion rate was 74 μl/s (Harvard pump, model No. 944; South Natick, MA). The perfusions lasted up to 3.5 min with 8 s added to allow the perfusate to completely fill the vascular space of the brain (12). The whole brain was immediately removed at the end of the perfusion and the right cortex was isolated, weighed and counted in a gamma counter. The brain to blood ratio (ml/g) was plotted against time (min) and the slope (K_i; the unidirectional influx) of rats perfused with ^{125}I-LHRH alone was compared with that of rats perfused with ^{125}I-LHRH and various unlabeled substances. These unlabeled substances were LH (2 and 10 nmol/ml), FSH (10 nmol/ml), β-estradiol (100 nmol/ml) and progesterone (100 nmol/ml).

RESULTS

Brain to Blood Studies

Intracerebroventricular injections of ^{125}I-LHRH. In the first two experiments testing brain to blood transport, the residual counts in the whole brains of mice injected ICV with 25,000 cpm/mouse ^{125}I-LHRH alone were compared with those in the whole brains of mice injected with ^{125}I-LHRH and the unlabeled pituitary hormones, FSH and LH. A one-way analysis of variance (ANOVA) found no statistically significant difference among the groups (Table 1).

TABLE 1

EFFECTS OF VARIOUS REPRODUCTIVE HORMONES ON BRAIN TO BLOOD TRANSPORT OF ^{125}I-LHRH (± SEM)

	% of Baseline Transport
Experiment 1:	
^{125}I-LHRH Alone	100 ± 8
+ unlabeled FSH (10 pmol/mouse)	112 ± 8
+ unlabeled FSH (1000 pmol/mouse)	97 ± 9
Experiment 2:	
^{125}I-LHRH Alone	100 ± 8
+ unlabeled LH (100 pmol/mouse)	88 ± 7
+ unlabeled LH (1000 pmol/mouse)	90 ± 9
Experiment 3:	
^{125}I-LHRH Alone	100 ± 8
+ unlabeled β-estradiol (1 pmol/mouse)	83 ± 9
+ unlabeled β-estradiol (100 pmol/mouse)	124 ± 15
+ progesterone (1 pmol/mouse)	95 ± 12
+ progesterone (100 pmol/mouse)	100 ± 8

No significant differences were found. An average of 8 animals was used per group.

In the third experiment, the effects of 25,000 cpm/mouse of ^{125}I-LHRH injected alone were compared with those of ^{125}I-LHRH injected together with the unlabeled steroid hormones, β-estradiol (1 or 100 pmol/mouse) and progesterone (1 or 100 pmol/mouse). A one-way ANOVA found no statistically significant difference among these groups either (Table 1).

Blood to Brain Studies

Intracarotid perfusion of ^{125}I-LHRH. In the experiments testing blood to brain transport, the effects of β-estradiol or progesterone in rats perfused with ^{125}I-LHRH alone ($K_i = 7.77 \times 10^{-3}$; $n = 4$, $r = .84$) were not significantly different from the effects in rats perfused with ^{125}I-LHRH and β-estradiol ($K_i = 8.19 \times 10^{-3}$; $n = 4$, $r = .76$) or in rats perfused with ^{125}I-LHRH and progesterone ($K_i = 8.47 \times 10^{-3}$; $n = 4$, $r = .94$).

In the experiments testing the effects of LH on the transport rate of ^{125}I-LHRH into the brain, the K_i for rats perfused with ^{125}I-LHRH alone was 5.31×10^{-3} ($n = 4$, $r = .62$). For rats perfused with ^{125}I-LHRH and LH (2 nmol/ml), the K_i was 4.68×10^{-3} ($n = 4$, $r = .93$) and for rats perfused with ^{125}I-LHRH and LH (10 nmol/ml) the K_i was 5.30×10^{-3} ($n = 5$, $r = .88$). Rats perfused with ^{125}I-LHRH and FSH (10 nmol/ml) had a K_i of 5.93×10^{-3} ($n = 5$, $r = .79$). No statistically significant difference was found among the slopes nor the intercepts with LH or FSH (Table 2).

DISCUSSION

A previous study has shown bidirectional saturable transport of ^{125}I-LHRH across the BBB (5). Such a system may explain how peripherally administered LHRH can exert actions on the brain (6, 9, 11).

It was not known, however, whether this transport system was related to the release of LHRH into the portal-hypophyseal blood. This release of LHRH from the hypothalamus is under the control of reproductive hormones. Thus an effect of these hormones on transport might indicate that release of LHRH into the blood from the median eminence was related to the transport of LHRH across the BBB.

No difference in the brain to blood transport of LHRH was demonstrated by ICV injections of ^{125}I-LHRH with LH, FSH,

TABLE 2

EFFECTS OF VARIOUS REPRODUCTIVE HORMONES ON BLOOD TO BRAIN TRANSPORT (K_i) OF ^{125}I-LHRH

	K_i (ml/g·min)
Experiment 1:	
^{125}I-LHRH Alone	7.77×10^{-3}; n=4, r=.84
+ unlabeled β-estradiol (100 nmol/ml)	8.19×10^{-3}; n=4, r=.76
+ unlabeled progesterone (100 nmol/ml)	8.47×10^{-3}; n=4, r=.94
Experiment 2:	
^{125}I-LHRH Alone	5.31×10^{-3}; n=4, r=.62
+ unlabeled LH (2 nmol/ml)	4.68×10^{-3}; n=4, r=.93
+ unlabeled LH (10 nmol/ml)	5.30×10^{-3}; n=4, r=.88
+ unlabeled FSH (10 nmol/ml)	5.93×10^{-3}; n=5, r=.79

No significant differences were found.

β-estradiol, or progesterone as compared with ICV injections of ^{125}I-LHRH alone. Blood to brain studies also found no difference in the entry rate of ^{125}I-LHRH given alone or with the unlabeled hormones β-estradiol, progesterone, LH or FSH.

The findings that reproductive hormones do not affect BBB transport of LHRH make it unlikely that the two systems are the same, which would agree with the anatomical evidence. Although the median eminence is part of the brain, its BBB is located at the ependymal layer (also termed the blood-cerebrospinal fluid barrier) that separates it from the rest of the brain and not at the vascular endothelial layer that would ordinarily separate it from the blood (8). Therefore, LHRH released from the median eminence would have to cross a BBB to enter the rest of the brain. Hormonally controlled release of LHRH from this region, however, does not require transport across the BBB to enter the blood.

The lack of effect of the hormones LH, FSH, β-estradiol, and progesterone demonstrates the independence of transport from reproductive hormonal production. It is possible that the transport system may be involved in those other functions ascribed to LHRH, such as effects on sexual behavior.

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