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Brain Uptake and the Analgesic Effect of Oxytocin - its Usefulness as an Analgesic Agent

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To establish the usefulness of oxytocin (OT) as an analgesic for women in delivery, the pharmacokinetic parameters and blood-brain barrier (BBB) permeability of [^{3}H]OT were obtained using an intravenous injection technique or the internal carotid artery perfusion/capillary depletion (ICAP/CDM) method. Brain uptake of OT was similar to that of sucrose, plasma space marker, indicating that OT has a poor BBB permeability. Moreover, the analgesic effects of OT injected through the jugular vein on nociception were evaluated by the tail-flick method. The antinociceptive effects of OT injected at a dose of 0.2 mg/kg or 2 mg/kg were dose-dependent. In addition, the analgesic effects of OT on the CNS were unaffected by naloxone, a m-receptor antagonist. In a similar manner to the opioid system, OT may play a modulatory role in antinociception.

Key words: Oxytocin, Brain uptake, Analgesic effect, Naloxone

INTRODUCTION

Oxytocin (OT) is a neurohypophyseal nonapeptide, which plays an important role in CNS function as well as in uterine contraction during delivery (North *et al.* 1993). Furthermore, recently it has been reported that OT may also have analgesic effects (Lunderberg et al. 1994; Yang, 1994), and it has been found that the release of OT is controlled by opioid receptors, especially κ and μ (Bert *et al.* 1991). Since oxytocin possesses analgesic activity in women in labor, and is thought to interact with opioid receptor, it appears reasonable to hypothesize that the drug may cross the blood-brain barrier (BBB) and, thereby, exert its analgesic activity. Despite this possibility, however, it is currently unclear whether the drug may be permeable to the brain. Oxytocin is a nonapeptide and opioid peptides generally do not cross the blood brain barrier (BBB) (Cornford *et al.* 1978; Houghten *et al.* 1980), except some of the dynorphins (Terasaki *et al.* 1989) or DALDA (Samii *et al.* 1994). This observation suggests that the central nervous system (CNS) is not involved in the pharmacological activity. Some studies have shown that OT has the effect of inhibiting tolerance to morphine (Kovacs *et al.* 1985a), heroin (Kovacs *et al.* 1985b) and opioid peptides like β-endorphin (Kovacs and Telegdy, 1987). Unfortunately, the brain permeability of oxytocin has not been reported in the literature, and it is still not clear whether peptides cross the BBB.

Therefore, the objective of this paper was to examine whether the drug can cross the BBB. We evaluated BBB permeability of OT as an analgesic acting on the CNS by means of the intravenous injection technique and the internal carotid artery perfusion/capillary depletion (ICAP / CDM) method (Partridge, 1984, Triguero *et al.* 1990). In addition, we examined the analgesic effects of OT administered with and without naloxone to determine if OT is associated with the opioid mechanisms.

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MATERIALS AND METHODS

Materials

^{3}H-labeled [Tyr-2, 6] oxytocin with a specific activity of 30 Ci/mmol and [^{14}C]sucrose were purchased from Du Pont-NEN (Pukyung Co. Seoul). Unlabeled oxytocin acetate salt, naloxone and all other reagents were purchased from Sigma Chemical (Seoul, Korea). A reverse-phase column (CLC-ODS; 5 μm, 4 μm × 24 cm) and a guard column (CLC G-ODS; 5 μm, 4 μm × 10 μm) were obtained from Shimadzu (Dongil Science, Korea). A tail-flick analgesia meter (model 33) was purchased from IITC Life Sciences (Jae Sae Yang Heng Co., Korea). Male Sprague-Dawley rats were provided by Samyuk Experimental Animals (Osan, Korea).

Brain permeability

In order to determine pharmacokinetic parameters and the BBB permeability-surface area (PS) product of the [^{3}H]oxytocin, a previously developed method was used (Kang Y. S. and Kim J. M., 1999). The intravenous injection (5 μCi, 23 ng as OT) followed by the internal carotid artery perfusion (1.5 μCi/min, 6.8 ng/min as OT; flow rate 1.0 μl/min) were carried out on ketamine anesthetized rats, as described previously (Kang Y. S. and Kim J. M., 1999). Plasma samples were obtained at 0.25, 1, 2, 5, 15, 30 and 60 min. At the end of the plasma sample collection, rats were sacrificed by decapitation and the brain was immediately removed. A portion of brain sample was weighed and processed for OT determination by liquid scintillation counting or HPLC analysis. The BBB PS product, which is equivalent to the rate of organ clearance, was determined for [^{3}H]oxytocin and [^{14}C]sucrose as follows (Bickel, 1993),

$$PS = \frac{(V_D - V_0)\cdot C_P(T)}{\int_0^T C_P(T)\cdot dt}$$

where $C_p(T)$ is plasma concentration at time T. V_0 of 10 μl/g (Yoshikawa, 1992) was used in the computation of the PS product for [^{3}H] oxytocin and [^{14}C]sucrose. The brain delivery or percent ID per gram of brain at T=60 min after intravenous injection was determined as follows:

$$\frac{\%ID}{g} = PS \times AUC(T)$$

$$AUC(T) = \int_0^T C_p(t)\cdot dt = \frac{A_1\cdot(1-e^{-k_1\cdot T})}{k_1} + \frac{A_2\cdot(1-e^{-k_2\cdot T})}{k_2}$$

Plasma extraction and HPLC analysis

The metabolism of [^{3}H] oxytocin in blood was evaluated by extraction of plasma samples and separation of the sample with the reverse-phase HPLC (Rabenstein, 1994). Plasma samples were obtained 0.25, 1, 2, 5, 10, 30, 60 min after intravenous injection of 23 ng of OT (5 μCi). The extraction method used in this laboratory was a modification of the extraction method used for vasopressin (LaRocelle, 1980). Plasma was pooled from three rats. An aliquot of pooled plasma (200 μl) was acidified with 20 μl 1N HCl and extracted with the C_{18} Sep-Pak (Waters Associates) column. Sep-Paks were prewashed with 5 ml methanol, 10 ml deionized water and, then, 10 ml of 0.1% trifluoroacetic acid (TFA). Samples were individually applied at a rate of 1 ml/min, and eluted from a column. The column was washed with 10 ml 0.1% TFA, and the sample was eluted with 4 ml 75% acetonitrile (ACN) in 0.1% TFA. The first and second radioactive fractions of effluent were combined and dried under vacuum in a speed vac. The sample was reconstituted in the HPLC mobile phase, 18% ACN in 0.1% TFA and a 100 ml aliquot was applied to a reverse phase column (4.6 mm × 25 cm; C18 ODS column). Isocratical elution was performed at a rate of 1 ml/min. The eluent was monitored spectrophotometrically at 220 nm for 25 min. One milliliter fractions were collected and counted by liquid scintillation counter. Typical recovery of this method was approximately 40% for OT.

Analgesia testing

Male Sprague-Dawley rats, weighing between 250 and 300 g, were anesthetized with ketamine-xylazine. A PE-10 catheter was inserted into the jugular vein and exteriorized through the back of the neck. After 24-48 h of recovery, the animal was adapted to plastic holders for 30 min, and baseline tail-flick latencies were determined at 15 min intervals. The beam setting was chosen to yield a baseline latency of 3-4 s, and the cutoff time was 10 s. Unlabeled oxytocin (dissolved in 0.9% saline, pH 7.4) was then injected through the jugular vein catheter at the dose of 0.2 or 2 mg/kg. The cannulae was flushed with 100 μl of normal saline (Bickel, 1994, Samii, 1994). When it was necessary, naloxone was administered at a dose of 10 mg/kg s.c. immediately after the second base-line measurement. Tail-flick analgesia was then measured for 60-75 min after injection of OT.

RESULTS

Pharmacokinetics and brain permeability

The plasma clearance of net [^{3}H]oxytocin (OT), intact [^{3}H]OT and [^{14}C]sucrose obtained by HPLC analysis is shown in Fig. 1. This demonstrates that OT is metabolically unstable. The pharmacokinetic analysis of this plasma clearance data is shown in Table I. After HPLC analysis, the area under the plasma concentration curve (AUC) of OT decreased to less than 20% and the total plasma clearance (Clt) of OT increased by a factor of five. The AUC of OT was about three times lower than that of [^{14}C]sucrose. The blood-brain barrier (BBB) permeability surface area product (PS) for [^{3}H]OT (1.09 ± 0.15 μl/min/g) was approximately three times greater than that of [^{14}C]sucrose (0.38 ± 0.08 μl/min/g). Correspondingly, the brain delivery for [^{3}H]OT (0.018 ± 0.003%ID/g) was similar to that of [^{14}C] sucrose (0.017 ± 0.002%ID/g), plasma space marker (Fig. 2). The reverse-phase HPLC separation followed by liquid scintillation detection of plasma samples (0.25 and 60 min after [^{3}H]OT injection) and the injectate are shown in Fig. 3. Intact OT was readily observed in the HPLC separation for 0.25 min plasma sample and the injectate. However, the peak was nearly disappeared for 60 min plasma sample. Thus, we conclude that OT is

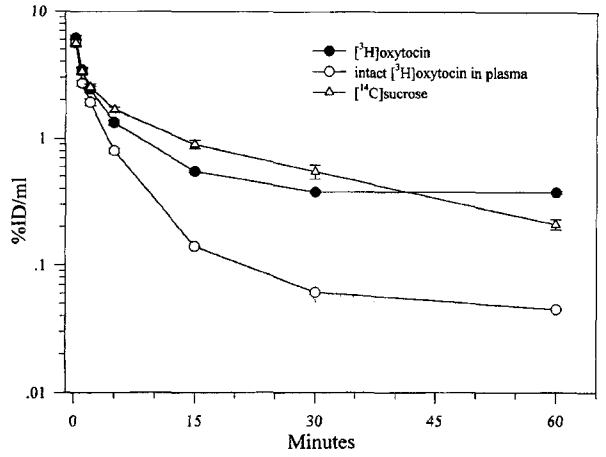

Fig. 1. Percent injected dose (ID) per ml. of plasma for [^{3}H]OT (closed circles) corrected [^{3}H]OT by HPLC (open circles) or [^{14}C]sucrose (triangles) in anesthetized rats vs. time after a single intravenous injection. Values are expressed as means ± SEM (n=3).

Table I. Pharmacokinetic parameters obtained using the intravenous injection technique

	[^{3}H]oxytocin	corrected [^{3}H]oxytocin	[^{14}C]sucrose
A_1 (%ID/ml)	4.73 ± 0.26	3.91 ± 0.24	4.36 ± 0.39
A_2 (%ID/ml)	0.52 ± 0.03	0.10 ± 0.01	1.60 ± 0.16
k_1 (min1)	0.38 ± 0.01	0.33 ± 0.01	0.72 ± 0.08
k_2 (min1)	0.0063 ±0.0012	0.014 ± 0.002	0.035 ± 0.001
t1/2 (min)			
Distribution	1.83 ± 0.05	2.12 ± 0.04	0.99 ± 0.12
Elimination	116 ± 21	51.6 ± 7.7	20.0 ± 0.3
AUC_t (%ID/mi/ml)	97.9 ± 10.7	19.5 ± 0.3	52.4 ± 4.7
Vd_{ss} (ml/kg)	611 ± 54	670 ± 130	206 ± 16
Clt (ml/min/kg)	4.36 ± 1.43	21.4 ± 0.4	8.10 ± 0.80
MRT	146 ± 30	31.4 ± 6.2	25.6 ± 0.5

Values are expressed as means ± S.E. (n=3). [^{3}H]Oxytocin before or after HPLC analysis with intact oxytocin in plasma and [^{14}C]sucrose (measured at 60 min); A_1 and A_2, plasma radioactivity; k_1 and k_2, rate constants: t1/2, half-life; Vd_{ss}, steadystate volume of distribution; Clt, total plasma clearance; MRT, mean residence time; AUC, area under the plasma concentration curve; ID, injected dose.

metabolically unstable in plasma.

Internal carotid artery perfusion/capillary depletion method

The brain volume of distribution (V_D) of [^{3}H]OT was similar to that of [^{14}C]sucrose after a 5 min internal carotid artery perfusion (Table II). Capillary depletion analysis indicated that the pellet V_D for [^{3}H]OT was two-fold greater than that of [^{14}C]sucrose, owing to the binding properties of peptides to the microvascular pellet (Banks, 1991).

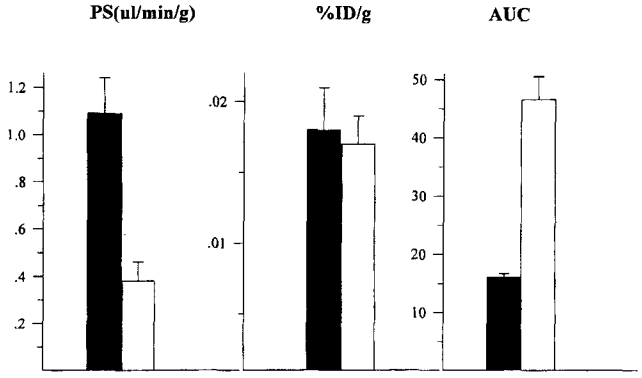

Fig. 2. Blood-brain barrier permeability surface area (PS) product, brain delivery measured as %ID/g of brain and AUC (%IDmin/ml), area under the plasma concentration curve at 60 min for [^{3}H]OT (closed bars) or [^{14}C]sucrose (open bars). Values are expressed as means ± SEM (n=3).

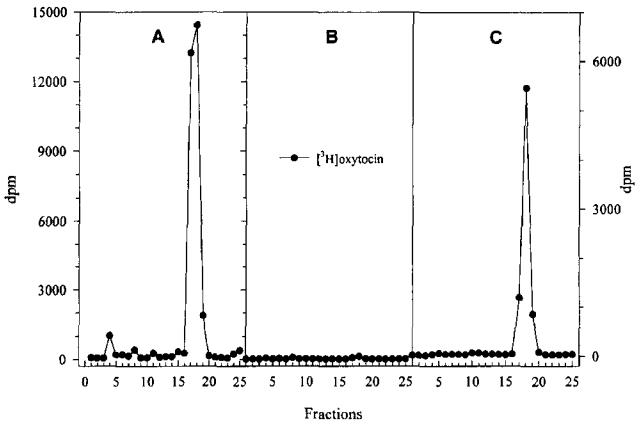

Fig. 3. Reverse-phase HPLC profile of extracts of plasma samples and injectate [^{3}H]OT before injection of tracer into rats and 0.25 min (B), and 60 min (C) after a single intravenous injection of [^{3}H]OT after extraction with C_{18} Sep-pak from acidified plasma.

Table II. Distribution volume of brain (VD) determined using the internal carotid artery perfusion/capillary depletion method

Perfusate	Solute	VD (µl/g)		
		Homogenate	Postvascula supernatant	Pellet
Oxytocin (34nM)	[^{3}H]Oxytocin	8.15 ± 0.21	7.58 ± 0.39	0.21 ± 0.06
	[^{14}C]Sucrose	8.06 ± 0.03	7.90 ± 0.31	0.10 ± 0.01

Values are expressed as means ± S.E. (n=3). Perfusion time is 5 min with a flow rate of 1.00 ml/min

Consequently, the brain uptake of [^{3}H]OT is comparable to sucrose, plasma volume marker. This observation indicate that OT does not cross the BBB.

Analgesia testing

The analgesia induced by a low dose (0.2 mg/kg) and a high dose (2 mg/kg) of OT was measured for 60-75 min after a single intravenous injection of the peptide in conscious rats. A little analgesia was observed after intravenous administration of the low dose, whereas an effective analgesia was induced by the high dose injection However, duration of the effect was short because of its rapid metabolism in plasma. Also, the analgesia of OT was not blocked by naloxone, a μ-antagonist (Fig. 4).

DISCUSSION

Oxytocin (OT), which has a molecular weight of 1007, has a BBB PS product that is threefold greater than that of sucrose (Fig. 2), as determined using an intravenous injection technique. Banks (1991) has evaluated the BBB permeability of radio-labeled peptides using the internal carotid artery perfusion technique and reported the PS value in the range of 1-5 μl/min/g (Banks, 1991). However, these PS values are unexpectedly high, since these peptides have low lipid solubility and are not expected to be transported across the BBB via carrier system. Therefore, radio-labeled amino acid, produced by the degradation of peptides in the peripheral tissue, may be transported across the BBB, thereby aretefactually resulting in the high BBB PS values for the peptides. Therefore, in without the separation of intact peptide from degraded amino acid in blood and brain tissue samples after the intravenous administration of radio-labeled neuropeptides, it is not possible to evaluate the extent to which a peptide actually undergoes transport through the BBB. This aspect of peptide, such as OT, pharmacokinetics requires HPLC analysis in combination with the intravenous injection technique. Indeed, we can readily confirm that OT is a metabolically unstable neuropeptide (Fig. 3). The plasma AUC of OT is three times lower than sucrose (Fig. 2, Table I), and because brain delivery is directly proportional to the plasma AUC, the brain uptake of OT is 0.018% ID/g, which is similar to that of sucrose (0.017 %ID/g).

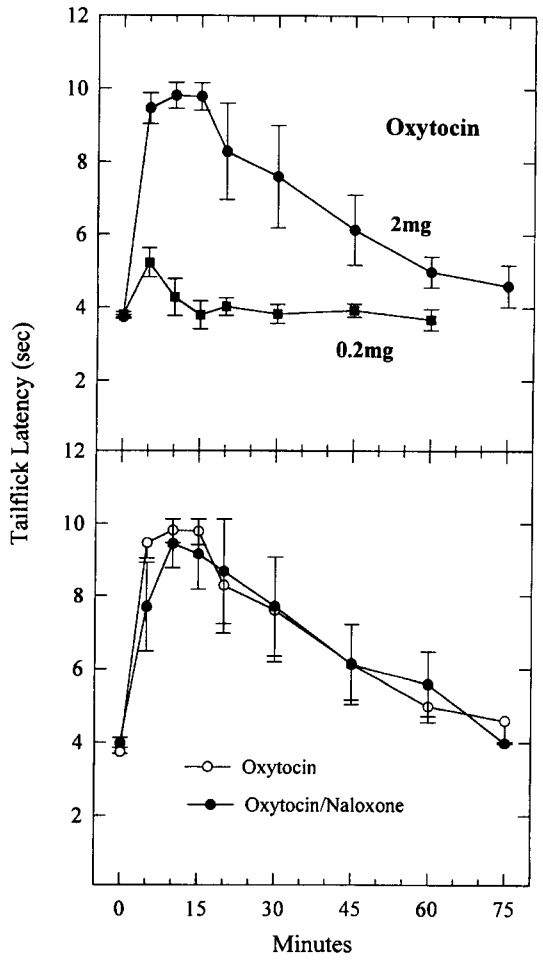

Fig. 4. Tail-flick latency after a single intravenous injection of 2 and 0.2 mg/kg of OT vs. time (upper panel). Base-line latencies were recorded 30 and 15 min before the i.v. injection. Naloxone was pretreated as a dose of 10 mg/kg s.c. immediately after the second base-line measurment (lower panel). Values are means ± SEM of three rats per group.

The present study shows that the BBB PS product for [^{3}H]OT after intravenous administration is 1.09 ± 0.15 μl/min/g (Fig. 2). This value is comparable to that of the DALDA, a dermorphin analogue (0.84 ± 0.13 μl/min/g), which has a molecular weight of 616 and a log PC value two-fold higher than that of sucrose. Recent studies have suggested that the lipid solubility of the solute may correlate with BBB permeability, providing the molecular weight of the solute is less than a threshold of 800-1000 (Pardridge, 1991). Therefore, OT may be transported across the BBB via the mechanism similar to DALDA (*i.e.*, diffusion through membrane pores of finite size into the brain).

The brain delivery of OT was approximately 0.018 %ID/g (Fig. 2) and an effective pharmacological response in brain was achieved with 2 mg/kg. Therefore, assuming the administration of OT at a dose of 2 mg/kg to a 0.25 kg rat, the brain concentration of OT is calculated to be 0.09 μg peptide/g brain. In the literature, the intrathecal administration of 0.5 μg/kg of OT in the rat (Yang, 1994) induces the central nervous system-mediated analgesia. These results suggest that therapeutic concentrations of OT may be achieved in brain after a systemic loading of a large dose. However, administration of high doses of OT may cause side effects, especially in the cardiovascular system. Therefore, a BBB drug delivery system, probably similar to that reported by Pardridge (1991), appears necessary to achieve analgesia without causing side effects. Our HPLC separation study suggests that a significant portion of OT is metabolized to smaller peptides. Therefore, the analgesic effect of OT may be partly derived from its

metabolites. Currently, this aspect of OT analgesia is not studied in the literature.

Anti-nociceptive effect of OT in rats is reported to be partly related to the activation of the descending inhibitory systems (Lunderberg *et al.* 1994). In this study, the OT induced was not blocked by naloxone, suggesting that, in addition to the opioid system, the oxytocinergic system has a modulatory role in nociception (Lunderberg *et al.* 1994). This aspect of OT analgesia is currently under investigation in our laboratory.

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