Dunlap, J., Brown, J. H. (1983) Mol. Pharmacol. 24: 15-22
Gilani, S. A. H., Cobbin, L. B. (1986) Naunyn-Schmiedeberg's Arch. Pharmacol. 332: 16-20
Marshall, R. J., McGrath, J. C., Miller, R. D., Docherty, J. R., Lamar, J.-C. (1980) Br. J. Anaesth. 52: 21S-32S
Nedoma, J., Dorofeeva, N. A., Tuček, S., Shelkovnikov, S. A., Danilov, A. F. (1985) Naunyn-Schmiedeberg's Arch. Pharmacol. 329: 176-181
Stockton, J. M., Birdsall, N. J. M., Burgen, A. S. V., Hulme, E. C. (1983) Mol. Pharmacol. 23: 551-557

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Twenty-one hormones fail to inhibit the brain to blood transport system for Tyr-MIF-1 and the enkephalins in mice

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Abstract—Tyr-MIF-1 (Tyr-Pro-Leu-Gly-amide) and methionine enkephalin are transported intact across the blood-brain barrier by a saturable, stereospecific system. This system has been found to be modulated by a few non-peptide substances and by certain conditions such as ageing and some stresses. We investigated the possibility that hormones structurally unrelated to Tyr-MIF-1 and the enkephalins might also be capable of modulating this transport. Twenty-one hormones were tested including steroids, proteins, glycoproteins, peptides, and thyroid hormones, in doses ranging from 0·01 pmol to 1 nmol/mouse by injecting each hormone directly into the lateral ventricle simultaneously with [^{125}I]Tyr-MIF-1. No clear effect on transport could be established for any of the substances at the doses tested. None of these substances seemed able to act as competitive inhibitors, to share their respective transport systems with Tyr-MIF-1, or to modulate immediately the saturable transport system.

Peptides, like other classes of hormones, can affect the central nervous system (CNS) in several ways including interaction with the blood-brain barrier (BBB). Peptides can cross the BBB (Kastin et al 1976; Banks & Kastin 1985a), alter the BBB transport of non-peptides (Tagliamonte et al 1976; Rudman & Kutner 1978; Goldman & Murphy 1981; Sankar et al 1981; Ermisch et al 1985), or have their BBB transport altered by non-peptides (Banks & Kastin 1986).

Some peptides cross the BBB by diffusing directly across the endothelial/ependymal membranes (Banks & Kastin 1985a, b). Other peptides are transported by saturable, carrier-mediated systems (Banks & Kastin 1984; Michals et al 1986). The best described of these saturable systems for peptides is the one that transports (Banks et al 1986a) Tyr-MIF-1 (Tyr-Pro-Leu-Gly-amide), an antiopiate (Kastin et al 1984, 1985; Galina & Kastin 1986), and methionine enkephalin (Tyr-Gly-Gly-Phe-Met), an opiate.

Although this system has very strict requirements as a transporter, it is regulated in an uncompetitive fashion by non-peptide substances (Banks & Kastin 1986) and altered by ageing (Banks & Kastin 1985c) and some stresses (Banks et al 1988). Thus, endogenous substances may be able to modify the transport system. Such possible modifiers could include hormones, substances known to regulate other systems and affect the CNS. We, therefore, examined many of the major ovarian, adrenal, testicular, hypothalamic, pituitary, and thyroid hormones for their ability to modify this transport system.

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Materials and methods

Transport was determined by the method previously described (Banks et al 1986). Briefly, male ICR mice (Blue Spruce Farms, Altamont NY), 17-20 g, were anaesthetized with ethyl carbamate (urethane). The skull was exposed and a hole 3·0-3·5 mm deep and 1·0 mm lateral and 1·0 mm posterior to the bregma was made into the left lateral ventricle with a guarded 26 gauge needle using a modified technique of Noble et al (1967). One μL of lactated Ringer's solution containing 25000 counts min^{-1} (5·6 fmol) of [^{125}I]Tyr-MIF-1 with or without candidate inhibitors was injected into the ventricle with a guarded Hamilton syringe (Hamilton Co, Reno, NV). Mice were decapitated 10 min after injection and the whole brain except for the pineal and pituitary counted in a gamma counter (Micromedic 4/200, Horsham, PA) for 3 min.

The results were expressed as a percent of the transport (%T) occurring in mice that received no candidate inhibitor, so that substances with no effect have a value of 100, substances with an inhibitory effect on transport have a value of less than 100, and substances with a stimulatory effect have values greater than 100. The equation used to derive %T was:

$$\%T = 100(A - Ex)/(A - Con)$$

where A is the amount of iodinated peptide available for transport, Ex is the amount of radioactivity remaining in the individual brains of mice that received candidate inhibitors, and Con is the mean amount of radioactivity remaining in the brains that received no candidate inhibitors.

The steroids oestrone, oestradiol, oestriol, progesterone, 17-hydroxyprogesterone, testosterone, dihydrotestosterone, dehydroepiandrosterone (DHEA) sulphate, and corticosterone were purchased from Sigma (St. Louis, MO) and injected in a lactated Ringer's solution containing 10% ethanol. Mice used as controls for this group also received 10% ethanol in their injectates. Thyroxine and triiodothyronine were also purchased from Sigma. Luteinizing hormone (LH), follicle stimulating hormone (FSH), growth hormone (GH), prolactin, thyroid stimulating hormone (TSH) and adrenocorticotrophin (ACTH) were a kind gift of the National Hormone and Pituitary Program of the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDKD). Luteinizing hormone releasing hormone (LHRH), thyrotrophin releasing hormone (TRH), angiotensin I, and angiotensin II were purchased from Bachem (Torrance, CA). LH, FSH, TSH, ACTH, LHRH, TRH, angiotensin I, and angiotensin II were dissolved in lactated Ringer solution. GH,

Table 1. Effect of varying doses of steroidal hormones on the brain to blood transport of [^{125}I]Tyr-MIF-1. Results are expressed as a percent of the baseline transport rate.

	Screening		
Compound	0·01 pmol (n)	10 pmol (n)	100 pmol (n)
Oestrone	69·3 ± 8·4 (5)	89·8 ± 8·6 (6)	117·5 ± 7·4 (5)
Oestradiol	85·7 ± 10·3 (6)	84·7 ± 10·1 (6)	82·0 ± 7·7 (6)
Oestriol	94·7 ± 8·4 (7)	91·1 ± 5·7 (6)	64·8 ± 7·8 (5)
Progesterone	105·1 ± 7·9 (7)	75·2 ± 15·4 (4)	69·0 ± 7·4 (6)
17-OH Progesterone	86·4 ± 13·1 (6)	111·2 ± 8·2 (6)	99·9 ± 12·4 (5)
Testosterone	53·9 ± 8·6 (7)	132·0 ± 5·6 (6)	99·9 ± 11·0 (6)
Dihydrotestosterone	98·5 ± 8·9 (7)	60·4 ± 15·6 (4)	109·2 ± 11·4 (6)
DHEA-Sulphate	80·6 ± 25·4 (4)	87·6 ± 8·4 (7)	70·6 ± 8·2 (6)
Corticosterone	112·5 ± 14·8 (5)	83·7 ± 9·9 (5)	74·1 ± 19·0 (7)

Presumptive inhibitors

Compound	(pmol)	% of baseline transport (n)
Testosterone	0·01	91·3 ± 14·4 (7)
Testosterone	10·0	94·6 ± 8·5 (7)
Dihydrotestosterone	10·0	108·8 ± 10·5 (7)
DHEA-Sulphate	10·0	106·1 ± 10·8 (7)
Corticosterone	10·0	97·7 ± 10·9 (7)

prolactin, thyroxine, and triiodothyronine were dissolved in a bicarbonate buffer (0·03 M $NaHCO_3$, 0·15 M NaCl, pH 10·8) according to the instructions of the NIDDKD. In all circumstances, control mice were injected with the corresponding buffer solution and solutions of hormones were used on the day of preparation.

Tyr-MIF-1 was labelled with ^{125}I using chloramine T and separated from unlabelled and di-iodinated peptide by high performance liquid chromatography. Specific activity was 500 nmol Ci^{-1}. Means are reported with their standard errors. Groups were compared with analysis of variance (ANOVA) and Duncan's multiple range test (DMRT).

Results

Table 1 shows the results for the steroids, expressed as a percent of the baseline transport rate, which was $0{\cdot}283 \pm 0{\cdot}017$ fmol g^{-1} min^{-1}. The overall ANOVA was significant: $F(27, 161) = 2{\cdot}85$, $P < 0{\cdot}01$. DMRT showed that testosterone at 0·01 pmol, but not at 10 pmol or 100 pmol, and dihydrotestosterone at 10 pmol, but not at 0·01 pmol or at 100 pmol, had transport rates that were statistically lower ($P < 0{\cdot}05$) than those of control mice. These substances at those concentrations were used again with testosterone, 10 pmol (which had the highest value suggestive of stimulation, $P = 0{\cdot}08$) and two substances that had previously shown no effect (Table 1, presumptive inhibitors). The baseline transport rate was $0{\cdot}260 \pm 0{\cdot}032$ fmol g^{-1} min^{-1}; ANOVA showed effect: $F(5,35) = 0{\cdot}355$, $P > 0{\cdot}25$.

Table 2 shows the effect of non-steroidal hormones on the transport of [^{125}I]Tyr-MIF-1. Because thyroxine, triiodothyronine, GH, and prolactin required different buffers and, therefore, different controls, they were analysed separately. For the other non-steroidal hormones listed in Table 3, the baseline transport rate was $0{\cdot}281 \pm 0{\cdot}018$ fmol g^{-1} min^{-1}. DMRT showed that only mice that had received LH at 1 nmol ($P = 0{\cdot}048$) had a transport rate that was significantly different from control mice. ANOVA showed no statistical differences for the mice receiving the control buffer ($0{\cdot}192 \pm 0{\cdot}016$ fmol g^{-1} min^{-1}), thyroxine, triiodothyronine, GH, or prolactin: $F(4, 42) = 1{\cdot}49$, $P > 0{\cdot}1$. LH was retested (Table 2, presumptive inhibitors) at 1·0 nmol with a larger number of mice along with FSH, the substance in this group with the highest transport value, even though this was not significantly different from control ($P = 0{\cdot}13$). Transport rate in the control mice was $0{\cdot}261 \pm 0{\cdot}035$ fmol g^{-1} min^{-1}) and ANOVA showed no significant effect on transport this time: $F(2, 38) = 1{\cdot}38$, $P > 0{\cdot}25$. GH and prolactin were also retested with a larger number of mice and, again, there was no significant effect: $F(2,43) = 1{\cdot}21$, $P > 0{\cdot}25$; control transport rate: $0{\cdot}216 \pm 0{\cdot}024$ fmol g^{-1} min^{-1}.

Table 2. Effect of non-steroidal hormones on the transport of [^{125}I]Tyr-MIF-1. Results are expressed as a percent of the baseline transport rate.

	Screening	
Compound	10 pmol (n)	1 nmol (n)
LH	79·8 ± 5·6 (5)	66·3 ± 1·6 (3)
FSH	120·0 ± 11·0 (5)	123·3 ± 6·4 (5)
TSH	113·8 ± 10·4 (7)	106·6 ± 10·8 (7)
ACTH	76·0 ± 13·9 (5)	72·0 ± 6·4 (6)
LHRH	89·8 ± 7·8 (5)	74·3 ± 8·2 (5)
TRH	75·1 ± 14·1 (6)	77·6 ± 7·2 (6)
Angiotensin I	81·1 ± 5·6 (5)	73·9 ± 16·3 (5)
Angiotensin II	84·1 ± 5·0 (7)	78·4 ± 11·6 (6)
GH	116·9 ± 10·2 (10)	
Prolactin	140·2 ± 13·4 (10)	
Thyroxine	115·7 ± 12·4 (10)	
Triiodothyronine	121·1 ± 11·5 (10)	
	Presumptive inhibitors	
Compound	10 pmol (n)	1 nmol (n)
LH		77·3 ± 8·4 (12)
FSH		93·4 ± 6·1 (13)
GH	119·7 ± 9·1 (18)	
Prolactin	97·2 ± 11·6 (18)	

Discussion

Although some investigators have interpreted the literature as indicating that peptides in general do not cross the BBB and have discounted the possibility of transmembrane diffusion for peptides (Pardridge et al 1981; Pardridge 1986), we have found that many peptides enter the CNS as a function of their lipophilicity (Banks & Kastin 1985a,b; Banks et al 1986b) to a degree that can alter CNS function (Miller et al 1986). Other peptides are transported across the BBB by carrier-mediated systems (Banks & Kastin 1984; Banks et al 1986a; Michals et al 1986), of which the best described transports Tyr-MIF-1 and the enkephalins from the CNS to the periphery (Banks & Kastin 1984; Banks et al 1986a). Transport rates determined by the method used here are based on residual counts in the brain, but agree well with rates based on the appearance of counts in the

circulation (Banks et al 1987), and can reflect the presence of a modifying substance.

The current studies largely confirm the specificity of this transport system for the small, *N*-tyrosinated peptides. None of the 21 hormones tested clearly altered transport at the doses tested. This further distinguishes this system from other transport systems that have been shown to transport substances out of the CNS. For example, thyroxine, which is transported out of the CNS by a saturable system (Banks et al 1985), is not inhibited by iodide, which is also transported from the brain to the blood (Davson & Hollingsworth 1973), or by Tyr-MIF-1. These studies now show that at the concentrations tested the thyroid hormones are also without effect on the transport of [^{125}I]Tyr-MIF-1.

These experiments also show the relative resistance of this peptide transport system to modification. Leucine and aluminium can alter transport in an uncompetitive and non-competitive fashion, respectively (Banks & Kastin 1986), and stress and ageing have also been shown to affect this system (Banks & Kastin 1985c, in press). Many of the hormones tested here can be influenced by opiates or can influence responses to opiates, but our results indicate that these modifications are not associated with appreciable changes in peptide transport.

The results, however, do not entirely exclude an action of the hormones on this transport system. These hormones were injected along with the [^{125}I]Tyr-MIF-1 into the lateral ventricle of the brain, a method that tests substances for immediate action such as occurs with competitive inhibitors or substances that act immediately on regulatory sites. However, a less accessible regulatory site might exist (for example, on the blood side of the BBB), the regulatory effect might be delayed, or the action on transport might be indirect requiring mediation through time consuming processes. Also, immediate effects might occur at different concentrations of the hormones than those tested, although the hormones were tested over a wide range, including levels that should have exceeded concentrations found in the CSF (Wood 1983). Overall, these studies demonstrate a high degree of specificity for the carrier-mediated system transporting Tyr-MIF-1 and the enkephalins and suggest that modulation of this system is limited to a relatively small number of substances.

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References

Banks, W. A., Kastin, A. J. (1984) Pharmacol. Biochem. Behav. 21: 943–946

Banks, W. A., Kastin, A. J. (1985a) Psychoneuroendocrinology 10: 385–399

Banks, W. A., Kastin, A. J. (1985b) Brain Res. Bull. 15: 287–292

Banks, W. A., Kastin, A. J. (1985c) Neurosci. Lett. 61: 171–175

Banks, W. A., Kastin, A. J. (1986) J. Pharmacol. Exp. Ther. 239: 668–672

Banks, W. A., Kastin, A. J., Michals, E. A. (1985) Life Sci. 37: 2407–2414

Banks, W. A., Kastin, A. J., Fischman, A. J., Coy, D. H., Strauss, S. L. (1986a) Am. J. Physiol. 251: E477–E482

Banks, W. A., Kastin, A. J., Coy, D. H., Angulo, E. (1986b) Brain Res. Bull. 17: 155–158

Banks, W. A., Kastin, A. J., Horvath, A., Michals, E. A. (1987) J. Neurosci. Res. 18: 326–332

Banks, W. A., Kastin, A. J., Nager, B. J. (1988) Neuropharmacology in press

Davson, H., Hollingsworth, J. R. (1973) J. Physiol. 233: 327–347

Ermisch, A., Ruhle, H. J., Landgraf, R., Hess, J. (1985) J. Cerebral Blood Flow Metabol. 5: 350–357

Galina, Z. H., Kastin, A. J. (1986) Life Sci. 39: 2153–2159

Goldman, H., Murphy, S. (1981) Pharmacol. Biochem. Behav. 14: 845–848

Kastin, A. J., Nissen, C., Nikolics, K., Medzihradsky, K., Coy, D. H., Teplan, I., Schally, A. V. (1976) Brain Res. Bull. 1: 19–26

Kastin, A. J., Stephens, E., Erhensing, R. H., Fischman, A. J. (1984) Pharmacol. Biochem. Behav. 21: 937–941

Kastin, A. J., Stephens, E., Zadina, J. E., Coy, D. H., Fischman, A. J. (1985) Ibid. 23: 1045–1049

Michals, E. A., Kastin, A. J., Banks, W. A. (1986) Clin. Res. 34:199A

Miller, L. H., Turnbull, B. A., Kastin, A. J., Coy, D. H. (1986) Sleep 9: 80–84

Noble, E. P., Wurtman, A. J., Axelrod, J. (1967) Life Sci. 6: 281–291

Pardridge, W. M. (1986) in: Cserr, H. F. (ed.) The Neuronal Microenvironment. Annals of the New York Academy of Sciences, Vol. 481, New York, pp 231–249

Pardridge, W. M., Frank, H. J. L., Cornford, E. M., Braun, L. D., Crane, P. D., Oldendorf, W. H. (1981) in: Martin, J. B., Reichlin, S., Bick, K. L. (eds) Neurosecretion and Brain Peptides. Raven Press, New York, pp 321–328

Rudman, D., Kutner, M. H. (1978) Am. J. Physiol. 234: E327–332

Sankar, R., Domer, F. R., Kastin, A. J. (1981) Peptides 2: 345–347

Tagliomonte, A., DeMonits, M. G., Olianis, M., Onali, P. L., Gessa, G. L. (1976) Adv. Exp. Med. Biol. 69: 89–94

Wood, J. H. (1983) in: Wood, J. H. (ed.) Neurobiology of Cerebral Spinal Fluid. Plenum Press, New York, pp 43–65