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Comparison of the Analeptic Potency of TRH, ACTH 4-10, LHRH, and Related Peptides[1]

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BISSETTE, G., C. B. NEMEROFF, P. T. LOOSEN, A. J. PRANGE, JR., AND M. A. LIPTON. *Comparison of the analeptic potency of TRH, ACTH 4-10, LHRH, and related peptides.* PHARMAC. BIOCHEM. BEHAV. 5: SUPPL. 1, 135–138, 1976. Various peptide hormones appear to exert behavioral and pharmacologic effects apart from their classical endocrine actions. Thyrotropin-releasing hormone (TRH), for example, antagonizes the sedation and hypothermia produced by barbiturate and other depressant drugs and de Wied has shown that ACTH 4-10, TRH, LHRH and certain related substances show some activity in inhibition of extinction of a pole-jumping avoidance response in the rat. These data provided the impetus for screening ACTH 4-10, LHRH, and related peptides for analeptic activity. ACTH 4-10 and ACTH 4-7 were inactive in antagonizing pentobarbital whether administered peripherally or centrally. ACTH 4-7 amide and 4-Met(O_2),8-D-Lys,9-Phe-ACTH 4-9 were active regardless of route of administration. LHRH and two tripeptide fragments (*p*Glu-His-Trp-NH_2 and *p*Glu-His-Phe-NH_2) showed analeptic activity only after intracisternal administration. Thus, some peptide fragments related to ACTH 4-10 and LHRH were shown to share to some degree the analeptic properties previously demonstrated for TRH.

ACTH peptides Thyrotropin releasing hormone Luteinizing hormone-releasing hormone Sodium pentobarbital

THE NEUROHUMORAL control of adenohypophyseal hormone secretion by hypothalamic releasing and inhibiting hormones is now a largely accepted hypothesis [14]. In recent years, there have been several reports of direct central nervous system effects of these peptide hormones, apparently distinct from their role in the hypothalamic-pituitary axis. These data have been recently reviewed [6, 7, 11, 12]. Thus, thyrotropin-releasing hormone (TRH), besides stimulating the release of thyrotropin and prolactin from the anterior pituitary, potentiates the behavioral effects of L-DOPA in intact, hypophysectomized, and thyroidectomized animals [9]. TRH is also a potent antagonist of the narcosis induced by barbiturates [10], ethanol [1], and other centrally acting depressants [6].

The pioneering work of de Wied has clearly demonstrated that adrenocorticotrophic hormone (ACTH), an anterior pituitary hormone, and ACTH-related peptide fragments exert marked effects on behavior which are not mediated via the adenohypophyseal-adrenocortical axis [2]. The central nervous system effects of ACTH and related fragments have been reviewed [3]. In recent work [4] ACTH 4-7 has been shown to be the smallest fragment with comparable activity of the entire molecule in causing inhibition of the extinction of an active avoidance response. Because luteinizing hormone-releasing hormone (LHRH) and TRH showed some activity in this behavioral paradigm and because of certain structural similarities between TRH and ACTH 4-7, we sought to determine if LHRH, ACTH 4-7, and related analogues were active in reversing pentobarbital-induced sedation and hypothermia.

METHODS

Adult, male Swiss-Webster mice (18–22 g) were purchased from Dublin Laboratories, Dublin, Virginia and housed in a controlled lighting animal facility with free access to lab chow and water. Mice were not used for experimental purposes for at least 7 days after arrival in order to eliminate nonspecific stress. In the first series of experiments, mice were given either TRH (1 mg/kg), the test substance at 1 or 5 times equimolar concentration of 1 mg/kg of TRH, or vehicle consisting of 0.9% saline solution, pH 7.5, intraperitoneally (IP). Two minutes, later, all animals received 50 mg/kg sodium pentobarbital (IP). Sleeping time was considered the interval between loss of the righting reflex after barbiturate treatment and recovery of the reflex. Rectal temperatures were recorded at 33, 66, and 99 min after pentobarbital injections with a thermistor probe.

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In the second series of experiments, intracisternal (IC) injection of TRH (10 μg), equimolar amounts of test substance, or 10 μl of vehicle was performed 10 min after barbiturate administration (50 mg/kg IP). Other experimental conditions were essentially the same as described above. Data were evaluated statistically by use of Student's *t* test (two-tailed).

Peptides tested were ACTH 4-10 (H-Met-Glu-His-Phe-Arg-Trp-Gly-OH); ACTH 4-7 (H-Met-Glu-His-Phe-OH); ACTH 4-7 amide (H-Met-Glu-His-Phe-NH_2); a substituted ACTH 4-9 fragment: 4-Met (O_2), 8-D-Lys, 9-Phe-ACTH 4-9 [H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-OH]; and two tripeptide fragments (*p*Glu-His-Trp-NH_2) and (*p*Glu-His-Phe-NH_2); TRH (*p*Glu-His-Pro-NH_2); and LHRH (*p*Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2).

RESULTS

The results of the first series of experiments (IP administration) are shown in Table 1. None of the other peptide hormones or fragments had a greater analeptic potency than TRH. The two tripeptide analogues of TRH studied were inactive as were ACTH 4-10 and ACTH 4-7. ACTH 4-7 amide and the substituted ACTH 4-9 fragment significantly shortened barbiturate-induced sleeping time and hypothermia. The antagonism by the latter compound was dose related.

The results of the second experimental series (IC administration) are illustrated in Table 2. As opposed to the results obtained from IP study, the central administration of the 2 tripeptide analogues significantly antagonized barbiturate-induced narcosis. ACTH 4-7 and ACTH 4-10 were inactive centrally as well as peripherally. ACTH 4-7 amide and the substituted ACTH 4-9 congener continued to show significant analeptic potency after intracisternal injection as well as intraperitoneal injection. LHRH was also found to be active after central administration.

DISCUSSION

These experiments have clearly shown that LHRH and some peptide fragments related to LHRH and ACTH 4-10 share to some degree the analeptic properties previously demonstrated for TRH. Table 3 is a summary of the behavioral [4] and the analeptic potencies (IP and IC) of TRH, LHRH, and ACTH related peptides. The latter two columns represent data presented in this report. There is no clear correlation between the behavioral and analeptic potencies of these compounds. There are some interesting observations that can however be made. All compounds that are active peripherally are also active centrally, but some compounds display activity only when injected centrally. The substituted ACTH 4-9 fragment, which is the most active compound in the pole jumping avoidance paradigm, has significant analeptic potency whether administered centrally or peripherally. This may be due to

TABLE 1

INTRAPERITONEAL ADMINISTRATION

Compound	Dose*	N	Regain righting Min ± SEM	33 min rectal Temperature ± SEM
pGlu-His-Trp-NH_2	Control†	20	107.7 ± 6.4	38.7 ± 0.24
	1	20	90.5 ± 6.7	29.4 ± 0.22
	5	20	95.6 ± 6.2	29.5 ± 0.22
pGlu-His-Phe-NH_2	Control	10	112.7 ± 9.4	29.1 ± 0.21
	1	10	89.9 ± 8.1	29.7 ± 0.28
	5	10	102.2 ± 5.9	29.4 ± 0.16
$ACTH_{4-10}OH$	Control	10	94.0 ± 7.3	30.4 ± 0.27
	1	10	85.9 ± 10.9	30.1 ± 0.29
	5	10	97.1 ± 7.7	30.4 ± 0.26
$ACTH_{4-7}OH$	Control	10	83.3 ± 6.4	29.0 ± 0.23
	1	10	66.3 ± 4.5‡	30.2 ± 0.31¶
	5	10	78.8 ± 10.2	30.6 ± 0.42
$ACTH_{4-7}NH_2$	Control	20	104.4 ± 5.9	29.0 ± 0.23
	1	20	74.4 ± 5.5·	30.2 ± 0.31¶
	5	20	72.1 ± 6.2	30.6 ± 0.42¶
$ACTH_{4-9}$[4-Met (O_2)	Control	20	116.0 ± 6.2	29.2 ± 0.18
8-D-Lys, 9-Phe]	0.1	10	84.1 ± 10.4‡	29.8 ± 0.27
	0.3	10	80.2 ± 5.2¶	30.2 ± 0.20¶
	1	20	74.0 ± 5.3	30.3 ± 0.26¶
	5	10	70.3 ± 6.5	30.2 ± 0.21§
TOTAL CONTROL		90	110.6 ± 3.09	29.2 ± 0.10
TOTAL TRH (1 mg/kg)		80	50.0 ± 1.96	32.8 ± 0.22

* = Dose equimolar to 1 mg/kg TRH.
† = Control is 0.9% NaCl, pH 7.5.
‡ = $p<0.05$ (Student's two tailed *t*-test).
§ = $p<0.02$.
¶ = $p<0.01$.
= $p<0.001$.

TABLE 2

INTRACISTERNAL ADMINISTRATION

Compound	N	Regain righting Min ± SEM	N	Control* Regain righting Min ± SEM	N	TRH Regain righting Min ± SEM
pGlu-His-Trp-NH_2	20	54.2 ± 2.7†				
			20	80.9 ± 4.7	20	34.8 ± 2.0
pGlu-His-Phe-NH_2	20	55.2 ± 2.2†				
$ACTH_{4-10}OH$	10	78.8 ± 9.9				
			10	91.6 ± 8.8	10	36.6 ± 2.8†
$ACTH_{4-7}OH$	10	97.8 ± 7.1				
$ACTH_{4-7}NH_2$	20	54.4 ± 3.5†				
			10	83.2 ± 4.1	10	41.9 ± 2.7†
$ACTH_{4-9}$ [4-Met (O_2) 8-D-Lys, 9-Phe]	20	54.9 ± 3.9†				
LHRH	20	49.0 ± 5.1†	10	86.7 ± 10.6	10	37.7 ± 2.8

All doses equimolar to 10 μg TRH.
*Control is 10 μl 0.9% NaCl, pH 7.5.
† = $p < 0.001$ (Student's two-tailed t-test).

TABLE 3

Compound	Relative value* in de Wied's Inhibition of Extinction of Pole Jumping Avoidance (Subcutaneous)	Relative value† in shortening pentobarbital induced sedation (Intracisternal)	Relative value† in shortening pentobarbital induced sedation (Intraperitoneal)
pGlu-His-Pro-NH_2 (TRH)	0.3	1.0‡	1.0‡
pGlu-His-Trp-NH_2	0.5	0.58‡	0.31
pGlu-His-Phe-NH_2	0.3	0.56‡	0.42
H-Met-Glu-His-Phe-Arg-Trp-Gly-OH ($ACTH_{4-10}$)	1.0	0.23	0.15
H-Met-Glu-His-Phe-OH ($ACTH_{4-7}$)	1.0	0.11	0.27
H-Met-Glu-His-Phe-NH_2	—	0.70‡	0.52‡
H-Met (O_2)-Glu-His-Phe-D-Lys-Phe-OH	1000.0	0.69‡	0.61‡
pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (LHRH)	1.0	0.77‡	—

*Values compared to $ACTH_{4-10}$ as the standard.
†Values compared to TRH as the standard and obtained from data presented in Table 1 and 2.
‡ = $p < 0.001$ (Student's two-tailed t-test).

this compound's increased resistance to biological degradation [15]. This is the only compound tested with analeptic activity that does not have an amide on the carboxy terminus of its polypeptide chain. Both ACTH 4-7 and ACTH 4-10 are missing this C-terminal amide, and both of these compounds were inactive regardless of route of administration. ACTH 4-7 regains analeptic properties in both methods of administration when an amide is substituted for the OH group on the carboxy terminus. Previous work from this laboratory has shown that TRH loses its analeptic potency when deaminated [13]. All compounds with activity in the reversal of pentobarbital-induced sedation contain a Glu-His dipeptide that is stabilized by another residue or by cyclization of the glutamate residue at the N-terminal end of the peptide chain and is protected by an amide group on the carboxyl end of the chain. Thus, TRH has a cyclized glutamate and C-terminal amide as does LHRH and the two tripeptide fragments. ACTH 4-7 amide is protected at both ends by an amide. LHRH is an antagonist to barbiturate if given intracisternally. LHRH is thought to be excluded from the central nervous system after peripheral administration [5]. The tripeptide *p*Glu-His-Trp-NH_2, is active centrally but not peripherally and contains the first three fragments of LHRH with a C-terminal amide group. The exclusion of LHRH from the CNS after peripheral administration may explain why this tripeptide is active only centrally. *p*Glu-His-Phe-NH_2 represents ACTH 5-7 with a cyclized glu-

tamate residue on the N-terminal side and an amide on the carboxy terminus. It, too, is effective in reversing barbiturate narcosis only when administered intracisternally. This specificity for route of injection may represent inability to survive breakdown in the periphery or inability to penetrate the blood-brain barrier.

Doses of these compounds needed to reverse barbiturate narcosis are much greater than the amounts needed to induce behavioral changes. For example, LHRH, when given to ovariectomized, hypophysectomized, estrogen-primed female rats induces lordotic behavior in the presence of a male after as little as 250 nanograms injected subcutaneously [8]. Considerably greater quantities of LHRH injected IC are however required to reverse the effects of barbiturate treatment. This discrepancy underlines the problem of comparing physiological and pharmacological effects

The molecular requirements for the analeptic properties of peptide fragments appear to be less rigid than their requirements for endocrinological activity. The requirement of some stabilizing configuration for a Glu-His dipeptide bond seems to be important. Characterization of the exact conformational elements that are important for this activity awaits further investigation.

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