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BRIEF COMMUNICATION

Growth Hormone Release Inhibiting Hormone: Neuropharmacological Studies

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PLOTNIKOFF, N. P., A. J. KASTIN AND A. V. SCHALLY. *Growth hormone release inhibiting hormone: neuropharmacological studies.* PHARMAC. BIOCHEM. BEHAV. 2(5) 693–696, 1974. – Significant potentiation of the behavioral effects of DOPA were observed in mice pretreated with GH-RIH. In addition, a slight reduction of oxotremorine induced symptoms was seen. No significant effects of GH-RIH were observed in several other tests involving the central nervous system (CNS). The results support our concept of the CNS actions of peptides.

Growth hormone DOPA Growth-hormone release inhibiting hormone Serotonin Hypothalamus
Oxotremorine Pituitary Seizures Central nervous system Aggression Behavior

A cyclic tetrapeptide has been isolated from ovine hypothalamic tissue [1] and shown to be active in man in inhibiting the release of growth hormone (GH) [5, 12, 13]. An occasional subject receiving this GH-release inhibiting factor (GH-RIH) reported vague symptoms suggestive of effects of the tetrapeptide on the central nervous system (CNS) [4]. The present studies utilized 7 different systems in an attempt to elucidate the CNS effects of GH-RIH by studying its actions on the behavior of mice.

METHOD

DOPA Potentiation (Potential Antidepressant Activity)

The potential antidepressant activity of GH-RIH was evaluated by observing its effects in the DOPA potentiation test in mice [3]. This test involves determination of the potentiation of motor responses of mice to a challenge dose of d,l-DOPA. Male, ICR mice (A.R. Schmidt, Sprague-Dawley) weighing 16–20 g were pretreated with a low dose of the monoamine oxidase inhibitor pargyline (40 mg/kg, orally). GH-RIH was then administered intraperitoneally followed by the d,l-DOPA (200 mg/kg, intraperitoneally). Four mice were used at each of 4 doses. The behavioral responses were rated as slight (1), moderate (2), or marked (3). Mice treated with an active antidepressant such as imipramine show a maximal behavioral response consisting of marked (3) increases in motor activity, jumping, squeaking, and irritability.

DOPA Antagonism (Potential Tranquilizing Activity)

Four mice at each of 3 dose levels were pretreated with pargyline (50 mg/kg, orally) 2 hr before receiving GH-RIH intraperitoneally. The mice were then challenged with d,l-DOPA (400 mg/kg, intraperitoneally). The effects routinely observed in unprotected mice are piloerection, salivation, rearing, irritability, and fighting. An active compound is defined as one which blocks the effects produced by this larger dose of DOPA. The degree of antagonism was scored as 0 (inactive), 1 (slight), 2 (moderate), and 3 (marked).

Serotonin Potentiation (Potential Antidepressant Activity)

The potential antidepressant activity of GH-RIH was

also evaluated in the mouse serotonin potentiation test. Known antidepressants such as imipramine have been reported to potentiate the central effects of serotonin as well as dopamine [2]. Three mice per dose were pretreated with pargyline (40 mg/kg, orally). The GH-RIH was then administered intraperitoneally at one of three doses, followed one hour later by a challenge dose of d,l-5-hydroxytryptophan (5-HTP), a precursor of serotonin, at a dose of 100 mg/kg, intraperitoneally. The behavioral effects observed were graded as slight (1), moderate (2), and marked (3), and included tremors, head movements, abducted limbs, and irritability.

Serotonin Antagonism (Potential Antipsychotic Activity)

Three mice at each of 3 dose levels were pretreated with pargyline (50 mg/kg, orally) 2 hr before receiving GH-RIH intraperitoneally. The mice were then challenged with 5-HTP (400 mg/kg, intraperitoneally). The effects observed in unprotected mice are tremors, head movements, abducted limbs and irritability. A compound active in this test blocks the effects produced by 5-HTP. The degree of antagonism was scored as 0 (inactive), 1 (slight), 2 (moderate), and 3 (marked).

Oxotremorine Antagonism Test (Potential Antiparkinsonian Agents)

Normal (ICR) male mice (16–20 g) were pretreated with varying doses of GH-RIH intraperitoneally one hour before administration of oxotremorine (0.5 mg/kg, intraperitoneally). The responses of the mice were recorded by observation techniques and were compared with those observed in mice which received only oxotremorine as a control [9]. The responses were graded as 0 (no effect), 1 (slight), 2 (moderate), and 3 (marked) for the effects of oxotremorine on each group of mice (four mice per group). The graded responses for the central effects of oxotremorine (including decreased motor activity, tremors, head twitch, limb abduction, and ataxia) were added together with the graded responses of the peripheral effects (lachrymation, salivation, and diarrhea) to produce a total score. Administration of oxotremorine itself results in an average total score of 22 out of a possible maximum total score of 24.

Audiogenic Seizures

A sensitive method of detecting potential anticonvulsant activity is the use of audiogenic seizures in mice [8]. Mice (O'Grady strain) specially bred for susceptibility to audiogenic seizures were used in these studies. Groups of 5 mice were pretreated with GH-RIH intraperitoneally 1 hr before testing in an auditory stress chamber, and were observed for convulsions. Mice not having convulsions were considered protected from the seizure effects.

Aggression Studies – Footshock-induced Fighting Behavior

The effects of GH-RIH on footshock-induced fighting behavior in mice were determined with a modified Tedeschi procedure [14] which has been widely used to evaluate the calming effects of drugs on aggressive behavior. Male, albino BALB/cJ mice (Jackson Laboratories) weighing 18–22 g were used. The mice were paired on an equal weight basis and each pair trained to fight in response to footshock. Only aggressive animals were selected. The paired mice were confined under a glass beaker (800 ml) placed on the grid floor of a Lehigh Valley fighting mouse apparatus. When electroshock was delivered to the grids, the mice responded with distinct and easily recognized aggressive and/or escape response. The presentation of footshock for each test session was limited to one minute. After the initial training period, the animals were tested immediately before (control) and at various time intervals after intraperitoneal administration of GH-RIH.

RESULTS

DOPA Potentiation and Antagonism

Significant potentiation of DOPA enhanced activity by GH-RIH was observed in a dose range of 1 to 4 mg/kg intraperitoneally. No antagonism of DOPA-induced hyperactivity was observed by GH-RIH in a dose range of 0.1 to 10 mg/kg. These results are shown in Table 1.

TABLE 1

DOPA TESTS 1 HR AFTER INTRAPERITONEAL GH-RIH

Test	Dose GH-RIH (mg/kg)	Behavioral Rating
Potentiation	0.5	1
	1	2
	2	3
	4	3
Antagonism	0.1	0
	1.0	0
	10.0	0

Serotonin Potentiation and Antagonism

No significant potentiation or antagonism by GH-RIH of serotonin activity was observed in a dose range of 0.1 to 10 mg/kg. These data are given in Table 2.

Oxotremorine Antagonism Test

No significant antagonism of oxotremorine induced symptoms was observed at a dose of 1 mg/kg intraperitoneally. However, a slight reduction of head twitch and body tremors was observed at a dose of GH-RIH of 10 mg/kg. This is shown in Table 3.

Audiogenic Seizure Test

No significant antagonism of audiogenic seizure by GH-RIH was observed at a dose of 10 mg/kg intraperitoneally. This lack of effect is illustrated in Table 4.

TABLE 2

SEROTONIN TESTS 1 HR AFTER INTRAPERITONEAL GH-RIH

Test	Dose GH-RIH (mg/kg)	Behavioral Rating
Potentiation	0.01	0
	0.1	0
	1.0	0
Antagonism	0.01	0
	0.1	0
	1.0	0

TABLE 3

OXOTREMORINE ANTAGONISM TEST 1 HR AFTER INTRAPERITONEAL GH-RIH

Dose GH-RIH (mg/kg)	Total Scores
1.0	22
10.0	16

TABLE 4

AUDIOGENIC SEIZURE TEST 1 HR AFTER INTRAPERITONEAL GH-RIH

Dose GH-RIH (mg/kg)	No. Convulsions
10	5/5
0 (controls)	5/5

Mouse Fighting Test

No significant reduction in fighting episodes was observed in mice pretreated with GH-RIH at a dose of 10 mg/kg. The results are shown in Table 5.

DISCUSSION

Earlier studies by Plotnikoff *et al.* [10,11] indicated that Pro-Leu-Gly-NH_2, an MSH-release inhibiting factor (MIF), and pyroGlu-His-Pro-NH_2, thyrotropin releasing hormone (TRH), are active in the DOPA potentiation test in mice. More recently, angiotensin II, in addition to MIF and TRH, was reported to be active in this test [6]. The present investigation shows that GH-RIH also potentiates the behavioral effects of DOPA.

These and other peptides differ in their relative potency in the DOPA potentiation test, with Pro-Leu-Gly-NH_2 being the most active [6,7]. TRH, on the other hand, is the most active peptide tried in the serotonin potentiation test. The use of many types of neuropharmacological tests indicates different patterns of actions of different peptides on the brain. An influence of peptides of neuronal transmission should be given consideration in the explanation of their effects in laboratory animals and man. In support of our original concept, the present study with GH-RIH adds another hypothalamic peptide to the growing list of naturally occurring hormones which affect the CNS.

TABLE 5

MOUSE FIGHTING TEST AFTER INTRAPERITONEAL GH-RIH

Dose GH-RIH (mg/kg)	Fighting Episodes 30 min	90 min
10	26	28
0 (controls)	27	27

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