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BRIEF COMMUNICATION

Effects of Growth Hormone on Brain Biogenic Amine Levels[1]

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STERN, W. C., M. MILLER, J. E. JALOWIEC, W. B. FORBES AND P. J. MORGANE. *Effects of growth hormone on brain biogenic amine levels.* PHARMAC. BIOCHEM. BEHAV. 3(6) 1115–1118, 1975. – The effects of IP administered bovine growth hormone (GH) on regional brain serotonin, 5-hydroxyindoleacetic acid (5-HIAA) and norepinephrine levels in rats were examined. GH decreased the levels of both monoamines and 5-HIAA in the diencephalon and brainstem while not affecting telencephalic concentrations. In hypophysectomized rats, however, GH produced significant elevations of monoamine and 5-HIAA levels in all brain regions. In normal rats the decreases in norepinephrine content produced by GH were correlated with a reduction in the stimulatory action of d-amphetamine on general activity levels. These results demonstrate that GH can affect brain biogenic amines and that these effects have behavioral consequences.

Growth hormone Biogenic amines Norepinephrine Serotonin 5-HIAA d-Amphetamine General activity levels Hypophysectomy

LITTLE consideration has been given to the possible role(s) of growth hormone (GH) in central nervous system functioning. Investigations in man have shown that GH is primarily secreted during the portion of the night accompanied by slow-wave sleep [3, 10, 14]. In a prior study in cats we examined the possibility that this sleep-related release of GH might alter subsequent sleep-waking patterns by administering GH and electrographically recording the post-GH vigilance patterns. An elevation of the occurrence of rapid eye movement sleep was observed for the first 3 hr after GH injection [12]. Pilot studies in two cats showed that this sleep change was accompanied by a decrease in norepinephrine and serotonin levels in various brain regions. The present report represents a more detailed investigation in rats of the neurochemical effects of GH on regional brain amine levels. In addition, a behavioral experiment is reported which assessed whether the GH induced change in brain norepinephrine levels was correlated with a change in the stimulatory action of d-amphetamine on general activity levels.

METHOD

General Procedure

Animals. Twenty-four adult male Sprague-Dawley rats, age 60–80 days, weighing 160–260 g and 10 adult hypophysectomized (HYPOX) males of the same strain and age, weighing 170 g, were employed in the neurochemical studies. All rats were supplied by Charles River Breeding Co., Wilmington, Mass. The amphetamine-general activity study employed 10 male rats (Sprague-Dawley), age 70 days, weighing about 230 g. The rats were housed in group cages, with ad lib access to food and water and with a 12:12 hour light:dark cycle (lights on at 0600 hr).

Drugs. Bovine GH (Calbiochem, San Diego, California) was dissolved in 0.9 percent saline and injected IP at a dose of 1.0 mg/kg. d-Amphetamine sulphate was similarly dissolved and was given IP at 0.5 and 2.0 mg/kg, as salt. The saline vehicle was the control injection for both the GH and amphetamine groups.

Neurochemistry procedures. Rats were sacrificed by guillotine at 1400–1600 hr and the brains were removed. The telencephalon (entire cerebral hemispheres), diencephalon (hypothalamus and thalamus) and the pons-medulla were dissected immediately and frozen in liquid nitrogen. Levels of serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and norepinephrine (NE) were measured spectrophotofluorometrically in each tissue sample according to the method of Maickel *et al.* [7] and Thompson *et al.* [15].

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Activity levels. Rats were placed on a 38 × 38 cm dimly illuminated platform for 4 hr (0900–1300 or 1300–1700 hr) with food and water available ad lib. The platform was mounted on springs and the vibrations produced by movement were electrically transduced by a phonograph cartridge. The output of the phonograph cartridge was amplified and passed through a voltage level discriminator. The pulsatile output of the voltage discriminator was counted as the measure of activity.

Specific Experiments

The first study assessed the effects of GH at 1.0 mg/kg IP, on regional brain 5-HT, 5-HIAA and NE levels at 15, 60 and 180 min (n = 4 per point) following GH injection. Comparisons were made with data from saline treated controls (n = 6).

Most prior work on the metabolic effects of GH employed HYPOX rats. We therefore assessed in a second study whether the effects of GH on regional brain 5-HT, 5-HIAA and NE levels would be the same in normal and in HYPOX rats. HYPOX rats were given IP injections of either saline (n = 5) or GH (n = 5) at 1.0 mg/kg and were sacrificed 3 hr later. Comparisons of effects on HYPOX rats were made with data from the saline and 1.0 mg/kg GH groups (normal rats) from the first study.

The third study examined the behavioral interaction between GH and the stimulatory effects of d-amphetamine on general activity levels in normal rats. The prior neurochemical studies showed that GH altered NE levels. Since the effects of amphetamine on activity are primarily mediated by brain catecholamine systems [1], it might be expected that GH modification of norepinephrine levels would affect the stimulatory action of amphetamine. Each of the 10 rats in this study received the following pairs of IP injections of saline (S), GH (1 mg/kg) or d-amphetamine (A) at 0.5 or 2.0 mg/kg in random order: S-S, S-A0.5, S-A2.0, GH-S, GH-A0.5, GH-A2.0. The first of each pair of injections was given 90 min after the start of the activity recording session. The second injection followed 30 min after the first. Activity was then recorded for 120 min after the second injection (amphetamine or saline) was given.

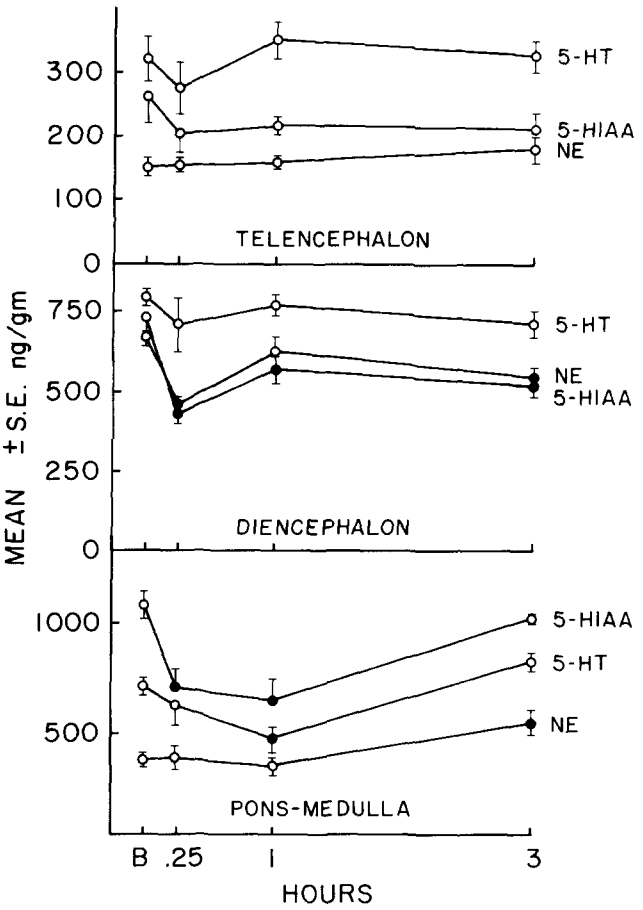

FIG. 1. Time course of effects of 1.0 mg/kg, IP, of bovine growth hormone (GH) on biogenic amine and 5-HIAA levels in regional areas of rat brain. The abscissa represents the time between injection of GH and sacrifice. Filled-in points indicate significant differences ($p<0.05$, 2-tailed) from the saline injected baseline (B) values. Nine one-way analyses of variance with post-hoc Dunnett t-tests (GH vs. baseline) were employed for the amine and 5-HIAA levels in the three brain regions [18].

RESULTS

The monoamine and 5-HIAA levels in the telencephalic samples were not changed by GH (Fig. 1). However, 5-HIAA and NE levels in the diencephalon were significantly depressed by 20–40 percent at most time points following GH with an incomplete recovery at the 3 hr post-GH time point. 5-HT levels were slightly but non-significantly depressed at all times after GH. In the pons-medulla, fewer effects of GH were observed. In this area 5-HIAA levels were generally decreased by 35–45 percent after GH, while no consistent changes in NE or 5-HT occurred. For example, 5-HT levels were lowered at 1 hr while NE was elevated at 3 hr. The overall trend in diencephalon and pons-medulla was that of lower amine and 5-HIAA levels after GH administration.

The neurochemical effects of GH in normal and HYPOX rats frequently differed. Table 1 shows that telencephalic 5-HIAA and NE levels were significantly increased by 60 percent after GH injection in HYPOX rats whereas these levels in GH treated normal rats were unaffected. Similarly, in the diencephalon 5-HT and 5-HIAA levels of the HYPOX rats were elevated by 50–80 percent after GH while in normal rats they were significantly decreased by 10–30 percent. However, GH lowered diencephalic NE in both normal and HYPOX rats. In the pons-medulla region GH had no effects in HYPOX rats whereas in normal rats 5-HT and NE levels were significantly increased.

The effects of pretreatment of normal rats with GH or saline on the stimulation of general activity levels by d-amphetamine are shown in Fig. 2. GH did not influence activity levels when 0 (saline vehicle) or 0.5 mg/kg of d-amphetamine were given. The 0.5 mg/kg dose of d-amphetamine with saline or GH pretreatment did not significantly stimulate behavior. In the saline pretreated group the 2.0 mg/kg dose of d-amphetamine significantly increased activity levels to about 6 times that of baseline values. GH pretreated rats injected with 2.0 mg/kg of d-amphetamine also exhibited enhanced activity levels, but to a significantly lower degree, i.e., to about 2–3 times baseline. Thus, GH significantly reduced the stimulatory effects of d-amphetamine.

TABLE 1

NORMAL VERSUS HYPOX RATS. EFFECTS OF GROWTH HORMONE (1.0 MG/KG, IP) ON REGIONAL BRAIN LEVELS OF 5-HT, 5-HIAA AND NE. RATS WERE SACRIFICED 3 HR AFTER SALINE OR GH INJECTIONS.

	Mean ± SE ng/gm			
	Normal Saline N = 6	Normal GH N = 4	Hypox Saline N = 5	Hypox GH N = 5
		Telencephalon		
5-HT	321 ± 35	327 ± 24	351 ± 31	355 ± 8
5-HIAA	266 ± 43	211 ± 30	182 ± 13	290 ± 18‡
NE	151 ± 14	181 ± 23	143 ± 6	224 ± 5§
		Diencephalon		
5-HT	795 ± 24	709 ± 38*	564 ± 48	835 ± 42‡
5-HIAA	753 ± 46	523 ± 31‡	663 ± 84	1207 ± 163†
NE	686 ± 21	535 ± 27§	849 ± 109	570 ± 84*
		Pons-Medulla		
5-HT	702 ± 40	833 ± 35*	837 ± 35	713 ± 59
5-HIAA	1093 ± 54	1016 ± 7	984 ± 59	1119 ± 120
NE	368 ± 14	567 ± 40§	555 ± 31	415 ± 71

Two-tailed t tests [18] comparing GH to saline values: *$p<0.10$ †$p<0.05$ ‡$p<0.01$ §$p<0.001$

DISCUSSION

The neurochemical study of the effects of bovine GH on regional brain 5-HT, 5-HIAA and NE levels show changes which are region dependent. Telencephalic areas were much less affected by GH than the diencephalon or pons-medulla. The predominant effect of GH in these latter two regions (in normal rats) was to lower brain monoamine and 5-HIAA levels by up to 50 percent, with 5-HIAA being somewhat more affected than 5-HT. The fact that the present monoamine changes were found in as little as 15 min following GH injection suggests that GH is rapidly taken up into brain tissue and quickly affects ongoing monoamine metabolism. This is supported by our observations (manuscript in preparation) that I^{125}-labelled GH is taken up into all brain regions of the rat. It is of course possible that the effects of GH on monoamine and 5-HIAA levels in brain were not due to direct action of GH on brain tissue but rather resulted from a non-specific change in amino acid availability due to the peripheral effects of GH. However, this interpretation is not supported by the present observations that the amine lowering effects of GH did not occur in all regions of the brain – the main effects occurred in diencephalic and pons-medulla sections, not in the telencephalon. It is likely that if the decreases in brain monoamine and 5-HIAA levels observed following GH were due to peripheral effects of GH, then amine levels in all brain areas should have been affected.

The present neurochemical results are among the first to demonstrate an effect of GH on the neurochemistry of the CNS in adult animals. Recently, Drucker-Colin *et al.* [2] have found that rat GH increased protein content of rat brain 2 hr after injection. Other studies have reported that GH increased brain protein synthesis in young hypothyroid rats [6] but not in adult hypophysectomized rats [5,13]. GH has also been found to affect biogenic amines in rat liver. Porcine GH increased polyamine (spermine) synthesis but concurrently decreased spermine levels by up to 30 percent in rat liver [4,8]. The decrease in liver spermine levels is similar to the present results of decreases in rat brain 5-HT and NE levels after bovine GH. However, spermine levels in the liver recovered to above normal levels at 3 to 4 hr following GH [8], whereas we observed incomplete recovery of amine levels. The mechanism by which GH altered brain monoamine levels in the present study is unclear. Since brain 5-HIAA levels were also decreased, it would seem that the lowered levels of 5-HT following GH were not produced by a reserpine-like release of 5-HT. Future studies of the dynamics of brain monoamines following GH should reveal the manner in which the presently observed change in 5-HT and NE levels occurred.

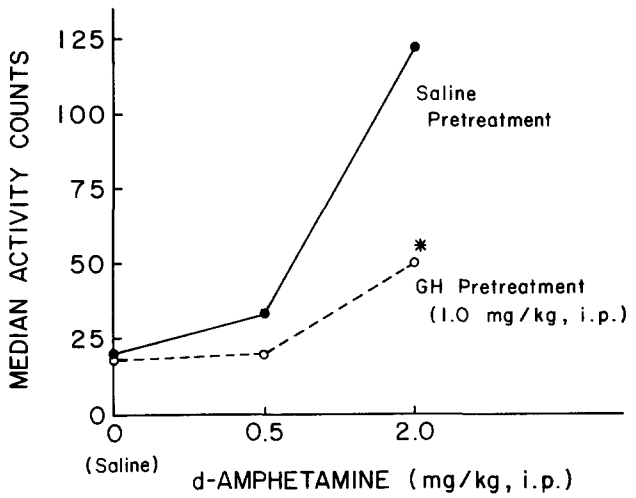

FIG. 2. Effects of growth hormone (GH) on the stimulation of general activity by d-amphetamine in normal rats. Rats were pretreated with saline or GH and were injected IP 30 min later with d-amphetamine at zero (saline vehicle), 0.5 or 2.0 mg/kg. The median activity count of the 10 rats in the 6 drug conditions represent the activity in the 120 min period following the second injection (saline or amphetamine). Three Wilcoxen Matched-Pairs Signed-Rank test [11] compared the effects of GH and saline pretreatment on the action of amphetamine. * $p<0.05$, 2-tailed, compared to saline pretreated rats given 2.0 mg/kg of d-amphetamine.

In contrast to the results of GH administration to intact rats, amine levels were often elevated when GH was given to HYPOX rats. Perhaps, the effects of GH on neural metabolism may depend upon the presence of additional pituitary-endocrine factors. This view is supported by the recent findings of Thorngren and Hansson [16] in which a marked synergy between the effects of GH and thyroxine on body growth was reported. The possibility arises that the results of prior investigations of the effects of GH in animals should be reexamined since most studies have employed hypophysectomized animals.

The GH induced reduction in the stimulatory action of d-amphetamine agrees with the lowering of hypothalamic NE (following GH) found in the neurochemical studies. Since the stimulation of behavior by amphetamine is largely a result of enhanced release and/or reduced catabolism of brain catecholamines [1], it is likely that decreasing brain NE by GH would reduce the effects of amphetamine. Other agents which disrupt catecholamine functioning similarly antagonize the action of amphetamine, e.g., α-methyl-tyrosine [17] or reserpine [9]. Thus, the GH-amphetamine interaction study and two prior reports of enhanced REM sleep following GH [2,12] demonstrate that GH administration can modify behavior. We are currently investigating to what extent physiological variations in secretion of endogenous GH can modulate behavior.

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