PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 159, 210–212 (1978)

Stimulation of Growth Hormone Release by Intraventricular Administration of 5HT or Quipazine in Unanesthetized Male Rats[1] (40316)

E. VIJAYAN,[2] L. KRULICH, AND S. M. McCANN

Department of Physiology, University of Texas Health Science Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75235

Although there is considerable evidence in favor of a stimulating role of the central serotoninergic system on the secretion of GH in man and nonhuman primates (1–4), little information is available in other species (5). Collu *et al.* (6) reported that intraventricular injection of serotonin stimulated GH secretion in rats anesthetized with urethane and that the effect was abolished by phenoxybenzamine, an α receptor blocker. Since experiments on animals in deep urethane anesthesia are opened to some criticism, we have investigated, in the present work, the effect of intraventricular administration of serotonin in unanesthetized unrestrained rats and compared them with the effect of intraventricular administration of the serotonin receptor agonist quipazine (7).

Materials and Methods. Adult male rats of the Sprague–Dawley strain (Simonsen Laboratories, Gilroy, California) were used. They were housed under controlled conditions of lighting (light on from 0500 to 1900 hr) and temperature (24 ± 1°) with free access to food and water. After 2 weeks of adaptation in our animal facility, a 23-gauge stainless-steel cannula was implanted into the third ventricle and 1 week later the animals were fitted with Silastic intravenous catheters as described earlier (8, 9). On the day of the experiment, usually 2 days after implantation of the intravenous cannulas, the animals were transferred in their cages into a quiet laboratory and polyethylene extension tubes (PE50, 12 in. long) filled with a solution of heparin in 0.9% NaCl were attached to the distal end of permanent iv cannulas. Thirty to sixty minutes later a preinjection blood sample (0.6–0.8 ml) was withdrawn; then the intraventricular injection was performed and postinjection samples (0.6–0.8 ml) were taken at 10, 30, and 60 min into heparinized syringes. The volume of each sample was replaced immediately after each bleeding by an equal volume of 0.9% saline.

The intraventricular injections were performed according to the procedure described earlier (8, 9): Serotonin (serotonin creatinine sulfate complex, Calbiochem) or quipazine maleate (gift of Miles Labs, Inc.) were freshly dissolved in 0.9% NaCl; the pH was adjusted to 5.5 and then administered into the ventricle in a volume of 2 μl. The dosage of 5HT is in terms of the free base. Controls for the 5HT-treated animals received 40 μg of creatinine sulfate while controls to quipazine were injected with 0.9% NaCl. In all cases the intraventricular injection was given over a period of approximately 60 sec.

In two experiments the animals were injected with serotonin receptor blocker, methysergide maleate (gift of the Sandoz Laboratories), 10 mg/kg ip, 60 min before the intraventricular administration of either 5HT or quipazine.

After centrifugation of the heparinized blood samples, plasma was collected and stored frozen at −20° until assay. Concentration of GH in the samples was determined by the NIAMDD radioimmunoassay system for rat GH.[3] All samples were measured in duplicates at two different dilutions. The results are expressed in nanograms per milliliter in terms of the RP-1 GH standard provided with the kit.

The statistical significance of the results was evaluated by the paired t test for sequential changes within the same group and by Student's t test for differences between two groups for a particular time.

Results and Discussion. Intraventricular injection of 5HT, 4 or 20 μg, caused a significant

[1] Supported by Grants AM10073 and HD09988.

[2] On leave of absence from Delhi University, India.

[3] Kits for determination of GH were provided through the NIAMDD–NIH Pituitary Hormone Program.

0037-9727/78/1592-0210$01.00/0

elevation of plasma GH levels, which was apparent at 10 min and persisted throughout the duration of the experiment (Table I). The GH response was related to the dose of 5HT in that the peak levels at 30 min were significantly higher in animals receiving 20 μg of 5HT than in the animals injected with 4 μg. Injection of creatinine sulfate to control animals did not influence plasma GH. Pretreatment of the rats with methysergide had no influence on the preinjection GH levels, but it completely abolished the GH-stimulating effect of intraventricular 5HT.

Quipazine also induced elevation of plasma GH. However, in comparison with the effect of 5HT, the secretory responses were delayed and a dose-related increase appeared only at 30 min after administration of the drug which persisted until the 60-min duration of the experiment (Table II). The stimulatory effect of quipazine was abolished by pretreatment of the animals with methysergide. Intraventricular administration of 0.9% NaCl in the group of controls had no effect on plasma GH.

We think that our results provide strong evidence that activation of the central serotoninergic system promotes secretion of GH in the rat. This conclusion is most directly supported by the GH-stimulating effect of intraventricular administration of 5HT. Suppression of the effect of 5HT with serotonin receptor blocker, methysergide, lends additional support to this conclusion.

There is considerable evidence that quipazine activates the central serotoninergic system (10–12) probably by a combination of several effects, which include activation of serotonin receptors, inhibition of serotonin reuptake by serotoninergic nerve terminals, and possibly enhanced release of serotonin (7, 13–15). It is, therefore, highly probable that the stimulation of GH secretion following intraventricular administration of quipazine originated in the activation of the central serotoninergic system. The similarity between the effect of 5HT and quipazine as well as the fact that the effect of both drugs was suppressed by methysergide also speaks for this conclusion.

Difficult to explain is our observation that the GH-stimulating effect of quipazine was

TABLE I. THIRD VENTRICULAR INJECTION OF SEROTONIN OR SYSTEMIC ADMINISTRATION OF METHYSERGIDE FOLLOWED BY INTRAVENTRICULAR SEROTONIN ON PLASMA GH LEVELS (NANOGRAMS PER MILLILITER OF PLASMA).

Treatment and dose	Preinjection	Time after injection (min)		
		10	30	60
Control: creatinine sulfate, 40 μg (7)[a]	33.2 ± 1.5	28.9 ± 4.0	30.8 ± 2.8	34.0 ± 2.0
Serotonin, 4 μg (4)	31.3 ± 3.2	51.4 ± 7.6*	54.6 ± 6.0*	55.6 ± 12.0*
Serotonin, 20 μg (8)	27.8 ± 4.4	54.7 ± 9.0*	103.6 ± 6.5**	55.6 ± 3.4*
Methysergide,[b] 10 mg/kg, + serotonin, 20 μg (5)	24.5 ± 4.6	17.5 ± 6.3	25.8 ± 3.2	38.7 ± 7.5

[a] Number of rats per group.
[b] Methysergide was given ip in a volume of 0.1 ml of saline 1 hr before third ventricular injection.
* $p < 0.05$ vs preinjection level.
** $p < 0.001$ vs preinjection level.

TABLE II. THIRD VENTRICULAR INJECTION OF QUIPAZINE OR SYSTEMIC ADMINISTRATION OF METHYSERGIDE FOLLOWED BY INTRAVENTRICULAR QUIPAZINE ON PLASMA GH LEVELS (NANOGRAMS PER MILLILITER).

Treatment and dose	Preinjection	Time After injection (min)		
		10	30	60
Saline (8),[a] 2 μl	27.0 ± 3.3	30.6 ± 1.1	29.8 ± 3.2	30.3 ± 0.8
Quipazine, 4 μg (5)	31.0 ± 1.5	27.2 ± 2.1	62.6 ± 8.9*	67.2 ± 4.1*
Quipazine, 20 μg (4)	33.5 ± 2.3	26.3 ± 2.3	105.4 ± 2.6**	80.6 ± 9.2**
Methysergide, 10 mg/kg, + quipazine, 20 μg (4)	30.6 ± 2.1	23.6 ± 1.2	26.0 ± 0.6	27.0 ± 0.6

[a] As in Table I.
[b] Methysergide was given as in Table I.
* $p < 0.05$ vs preinjection level.
** $p < 0.001$ vs preinjection level.

delayed as compared with the effect of 5HT. This delay is probably not caused by different pharmacodynamic properties of quipazine, because both drugs induce activation of prolactin secretion, attaining peak levels 10 min after intraventricular administration (unpublished results). It is, therefore, possible to speculate that quipazine, in addition to activation of the central serotoninergic system, may have a short-lasting effect of another kind which is inhibitory to GH secretion.

Our results obtained with the intraventricular administration of 5HT in unanesthetized free-moving animals confirm the earlier work of Collu *et al.* (6) on animals anesthetized with urethane. To our knowledge this is the first report on the GH-releasing effect of quipazine.

Summary. Intraventricular injection of 5HT (4 and 20 μg) in unanesthetized, unrestrained male rats fitted with permanent intrajugular cannulas for withdrawal of blood samples caused a dose-related elevation of plasma GH levels. Similar effects were also observed following intraventricular injection of the serotonin receptor agonist, quipazine. The GH-releasing effect of both drugs was abolished by a serotonin receptor blocker, methysergide. It is concluded that activation of the central serotoninergic system stimulates GH secretion in the rat.

The authors wish to thank Mrs. Shirlee Barnes for secretarial assistance.

1. Imura, H., Nakai, Y., and Yoshimi, T., J. Clin. Endocrinol Metab. **36,** 204 (1973).
2. Müller, E. E., Brambilla, F., Cavagnini, F., Peracchi, M., and Panerai, A., J. Clin. Endocrinol. Metab. **39,** 1 (1974).
3. Smythe, G. A., and Lazarus, L., J. Clin. Invest. **54,** 116 (1974).
4. Bivens, C. H., Lebovitz, H. E., and Feldman, J. M., N. Engl. J. Med. **289,** 236 (1973).
5. Martin, J. B., *in* "Frontiers in Neuroendocrinology" (L. Martini and W. F. Ganong, Eds.), Vol. 4, p. 129. Raven Press, New York (1976).
6. Collu, R., Fraschini, F., Visconti, P., and Martini, L., Endocrinology **90,** 1231 (1972).
7. Fuller, R. W., Snoddy, H. D., Perry, K. W., Ronsch, B. W., Mollay, B. B., Bymaster, F. P., and Wong, D. T., Life Sci. **18,** 925 (1976).
8. Vijayan, E., and McCann, S. M., Neuroendocrinology **25,** 150 (1978).
9. Vijayan, E., and McCann, S. M., Neuroendocrinology **25,** 211 (1978).
10. Rodriguez, R., Rojas-Ramirez, J. A., and Drucker-Colin, R. R., Eur. J. Pharmacol. **24,** 164 (1973).
11. Medon, P. J., Leeling, J. T., and Phillips, B. M., Life Sci. **13,** 685 (1973).
12. Samamin, R., Bernasconi, S., and Quattrone, A., Psychopharmacology **46,** 219 (1976).
13. Jacoby, J. H., Howd, R. A., Levin, M. S., and Wurtman, R. J., Neuropharmacology **15,** 529 (1976).
14. Green, A. R., Youdim, B. H., and Graham-Smith, D. G., Neuropharmacology **15,** 173 (1976).
15. Hamon, M., Burgoin, S., Enjalbert, A., Bockaert, J., and Hery, F., Nannyn-Schmiedebert's Arch. Pharmacol. **249,** 99 (1976).

Received June 9, 1978. P.S.E.B.M. 1978, Vol. 159.