BRAIN MONOAMINES PARTICIPATION IN THE CONTROL OF GROWTH HORMONE SECRETION IN DIFFERENT ANIMAL SPECIES

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A LARGE body of evidence has recently been accumulated for the involvement of brain monoamines in the control of growth hormone (GH) secretion. While there seemed to be sufficient proof of the existence of a dual neurohormonal control of the secretion of this hormone from the anterior pituitary exerted by specific GH-releasing (GRF) and inhibiting (GIF) factors (1), information was still scanty regarding the possible neural influences impinging on the neurosecretory structures responsible for the elaboration of the releasing factors. The introduction of a fluorescence method for the histochemical localization of the neurotransmitters (2) was a turning point in the understanding of their physiological role in the release of anterior pituitary hormones.

This article is an attempt to consisely review the present status of knowledge on the participation of central nervous system (CNS) dopamine (DA), norepinephrine (NE) and serotonin (5-HT) in the control of GH secretion in different animal species.

STUDIES IN RODENTS

The first neuro-pharmacological studies performed in the rat showed that drugs endowed with anti-adrenergic effects (reserpine, chlorpromazine, tetrabenazine, α-methyl-*m*-tyrosine, α-methyl-dopa) were capable of blocking the release of GH induced by insulin hypoglycemia (tibial plate bioassay) (3,4). Administration of NE and DA into the lateral ventricles of normal and hypophysectomized rats to circumvent the blood brain barrier to catecholamines (CA) (5) was followed by decreased GH levels in the anterior pituitary and by depletion of hypothalamic GRF.

Central injection of 5-HT was ineffective (6,7). When given intraventricularly (IVT), NE induced a depletion of pituitary GH activity at doses at which DA was ineffective; this finding suggested a role for NE as the synaptic transmitter for the release of GRF (7). Subsequent development of radioimmunological (RIA) techniques for the measurement of rat GH in the plasma (8), revealed discrepancies between RIA and bioassay (BA) data (9) and affected conclusions in neuropharmacological studies of GH regulation in the rat. In this connection, COLLU *et al.* (10) reported a reduction of plasma GH levels after IVT administration of DA and a highly significant rise following central injection of 5-HT. KATO *et al.* (11) observed a significant increase in plasma GH after systemic administration of either phentolamine, an α-adrenergic blocker, or isoproterenol, an adrenergic β-stimulant drug. No increases were detected in response to L-dopa, the immediate biological precursor of both DA and NE (12).

A recent re-investigation of the role of brain monoamines in the control of GH

secretion in the rat by using the RIA technique has been undertaken. (COCCHI *et al.*, paper in preparation). These studies assigned a negative role for DA but no confirmation could be obtained for the stimulatory action of 5-HT. This role seems, instead, to be played by NE. Thus, administration of FLA-63, a selective blocker of NE biosynthesis (13), lowered plasma RIA-GH, while central sympathectomy by 6-hydroxydopamine (6-OHDA) or systemic administration of α-methyl-*p*-tyrosine (α-MT), procedures which affect both the noradrenergic and the dopaminergic function (14,15), did not modify plasma RIA-GH. The previous findings on the stimulatory role of DA on GH release (BA method) might be accounted for the high rate of conversion of IVT-injected DA to NE present at hypothalamic level (16).

In mouse, a species which appears particularly refractory to GH-releasing stimuli (17), SINHA *et al.* (18) observed a dramatic decrease in the serum GH levels following systemic administration of either DA or L-dopa and reduced levels were also present after intraperitoneal injection of epinephrine (E). Conversely, central administration of 6-hydroxydopamine markedly increased circulating GH levels (MÜLLER *et al.*, unpublished data).

PIG, SHEEP AND DOG

Data on the involvement of brain amines in the regulation of GH secretion in the pig are rather scanty. In this species, in which the response to GH-releasing stimuli appears to be blunted, no rise in plasma GH has been reported following subcutaneous injection of 1 mg of epinephrine; equally ineffective in this context, was peripheral administration of NE (19). In the sheep, plasma levels of GH were constantly low during an intravenous infusion of E and the plasma GH response to arginine infusion was abolished. Plasma non-esterified fatty acid (NEFA) levels were greatly increased during the 3 hr period of epinephrine infusion (20). Intracarotid infusion of phenoxybenzamine, an α-adrenergic blocker, resulted in a late increase in plasma levels of GH in mature cyclic ewes and produced additive effects when combined with an arginine stimulus. Infusion of L-dopa by itself had no effect on plasma levels of GH, but suppressed the expected increase of GH due to arginine (21). In the dog, intravenous L-dopa administration induced instead, a brisk increase in growth hormone levels, which was inhibited by phenoxybenzamine given intraventricularly (22).

PRIMATES

Monkey

Single injections of large doses of epinephrine provoked a marked and prompt increase in the plasma GH levels of female rhesus monkeys (23).

Studies conducted in conscious fasted baboons showed that α-adrenergic blockade, resulting from intravenous infusion of phentolamine, significantly depressed GH secretion, while β-adrenergic blockade with propranolol and ganglionic blockade with trimetaphan were associated with a significant prompt rise in GH. The observed GH changes seemed not to be associated with alterations in plasma glucose or NEFA levels, although, lowered plasma NEFA and glucose levels were present following propranolol administration (24). Infusion of much smaller doses of phentolamine directly into the third ventricle or anterior hypothalamus evoked a similar fall in

TABLE 1.

Drug	Animal species	Route of administration	←Dosage←	Effect	References
Epinephrine	Monkey	i.v.	5–40 μg/kg	↑	23
	Human	s.c.	10 μg/kg	→	51
		i.v.	0·14 μg/kg/min	→	35
Norepinephrine	Monkey	IVT	1 μg/min/30 min	→	27
		microinjected into VMN	10 μg	↑	26
	Human	i.v.	0·05–0·2 μg/kg/min	→	36
Methyl-amphetamine	Human	i.v.	15 mg/5 min	↑	46
Dopamine	Monkey	IVT	5 μg	↓	27
L-Dopa	Human	p.o.	500–1000 mg	↑	30
Apomorphine	Human	s.c.	0·75–1·5 mg	↑	38
Phentolamine	Monkey	i.v.	0·013 mg/kg/min	↓	24
		IVT	1–5 μg/min/30 min	↓	25
	Human	i.v.	0·5 mg/min/75 min	inhibits L-dopa	32
		i.v.	0·5 mg/min/90 min	inhibits insulin hypoglycemia	33
		i.v.	0·5 mg/min/90 min	inhibits vasopressin	52
		i.v.	3·5 mg/50 min	inhibits propranolol + E	36
		i.v.	0·5 mg/min/120 min	no effect on GH sleep peak	53
		i.v.	0·5 mg/min/120 min	inhibits arginine	54
Propranolol	Monkey	i.v.	0·011 mg/kg/min	↑	24
	Human	i.v.	0·21 mg/kg/10 min	→	35
		i.v.	0·20 mg/kg/120 min	↑	34
		i.v.	0·15 mg/kg/few min	potentiates amphetamine	46
		i.v.	0·15 mg/kg/few min	potentiates insulin hypoglycemia	33
Propranolol + E	Human	i.v.	0·21 mg/kg/10 min 0·07–0·14 μg/kg/min/30 min	↑	35
Timoxamine	Human	i.v.	0·1 mg/kg	potentiates amphetamine	46
Haloperidol	Human	p.o.	2 mg	attenuates insulin hypoglycemia	42
Chlorpromazine	Human	p.o.	100 mg	↓	43
Serotonin	Monkey	microinjected into VMN	10 μg	→	26
	Human*			↑	44
5-hydroxytriptophan	Human	p.o.	150 mg	↑	45
Tryptophan	Human	p.o.	70 mg/kg	slight increase	Author's unpublished data

→ means no effect
↓ means stimulation
↑ means inhibition

* Patients with carcinoid syndrome

GH (25). To exclude the possibility that centrally-administered phentolamine was acting systemically to lower GH, the blocking agent was infused into the abdominal inferior vena cava. It was necessary to infuse a dose 2 to 4 times greater to lower GH significantly (25). These data suggested the existence in the CNS of the baboon of α-adrenergic receptors regulating GH secretion. It was gratifying to observe that NE microinjected into the ventromedial nucleus of the hypothalamus consistently elevated GH in conscious baboons. Micro injections of 5-HT in the same area did not elevate GH (26). In contrast to central administration of NE, intrahypothalamic infusion of DA lowered plasma GH, while NE and isoproterenol given systemically induced no change in the hormone level (27).

Human

In man the infusion of epinephrine in doses sufficient to produce significant hyperglycemia did not provoke GH secretion, nor blunt arginine-stimulated GH release (28,29). The poor penetrability of the blood–brain barrier by CA was circumvented by administering L-dopa, a drug that easily crosses the blood–brain barrier and increases brain levels of both DA and NE (12). The results obtained showed that moderate doses of L-dopa caused a significant rise in plasma GH levels in patients with parkinsonism, requiring gram amounts of the substance (30) as well in normal subjects (31). The stimulatory effect on GH secretion by L-dopa was not blocked by either oral or intravenous glucose (30), but was reduced or potentiated respectively, by the concomitant infusion of phentolamine (32) or propranolol (MASSARA and CAMANNI, unpublished results), stressing the involvement of adrenergic α and β-receptors in the regulation of GH secretion. In line with these findings, previous studies had shown that blockade of α-adrenergic receptors by phentolamine in man prevented the increase in plasma GH that follows insulin-induced hypoglycemia in normal subjects. In contrast, β-adrenergic receptors blockade by propranolol augmented the levels of GH in hypoglycemic subjects, although this was associated with increased hypoglycemia and a decrement in NEFA (33).

However, in this study neither α nor β-adrenergic blockade had a detectable effect in the absence of hypoglycemia, although a stimulant effect was reported for propranolol alone in Japanese subjects (34). Systemic infusions of propranolol combined with epinephrine resulted in increased GH levels (35). In this instance the stimulatory effect of epinephrine on plasma HGH in the presence of propranolol was the result of an unopposed α-receptor activity, since β-stimulation would be expected to inhibit GH secretion. Accordingly, the epinephrine-propranolol stimulation was blocked by phentolamine (36). In addition to L-dopa, apomorphine a direct stimulant of DA receptors (37) induced a rise of HGH (38), an effect which is compatible with a dopaminergic mechanism in the release of HGH. In this line are the observations that diethyldithiocarbamate, a blocker of NE biosynthesis (39) did not prevent the HGH rise due to L-dopa (GIORDANO, MINUTO, MARUGO, BARRECA, FOPPIANI, personal communication) and that haloperidol or chlorpromazine, two neuroleptic drugs mainly antagonistic to DA (40,41) blunted the insulin-induced HGH rise (42,43).

With regard to 5-HT, although increased GH levels have been found in plasma of patients with excessive 5-HT secretion due to the carcinoid syndrome (44) or following administration of the biological precursor, 5-hydroxytriptophan (45),

much more information is needed before its role in the GH-releasing mechanism(s) can be definitely assessed. In our hands, the stimulatory effects of large amounts of triptophan on HGH secretion appeared to be slight and rather erratic (MÜLLER, BRAMBILLA, CAVAGNINI, PERACCHI, PANERAI, unpublished results).

Table 1 deals with the effects of drugs altering brain monoamines on GH secretion in primates.

CONCLUDING REMARKS

The studies mentioned are without doubt compatible with the view of brain monoamines intervention in the process of GH secretion; however, some aspects of this neurohumoral control are still unclear and warrant further investigations. In the rat it seems probable that the two adrenergic neurotransmitters NE and DA exert a dual effect on the secretion of RIA-GH, the former having a stimulatory influence, being inhibitory the latter. This concept is in line with the demonstration of both GRF and GIF factors in the rat hypothalamus. In the mouse the central DA tone seems to be predominant : in fact, concomitant reduction of both NE and DA due to 6-OHDA, resulted in increased GH levels in this species. No major role seems to be played by 5-HT, although data are discordant in this regard.

In the pig and the sheep the available evidence is rather scanty and the results available, if ever, favour an inhibitory role for NE (phenoxybenzamine administration in the sheep). The proof presented for an inhibitory role of the adrenergic system in these species is questionable since it is mainly based on the effect of systemic administration of high doses of epinephrine, a drug which does not cross appreciably the blood–brain barrier and might be capable to induce the observed effects through the feedback action of increased NEFA levels. This same observation also applies to some of the results obtained in rodents or the human following systemic administration of this adrenergic compound. In the dog the few available data point to a stimulatory role of the adrenergic system in the control of GH.

The same dual role for NE and DA present in the rat seems to extend to the monkey, a species in which the stimulant action of NE on GH release is particularly well documented as well as the inhibitory effect of α-adrenergic blockers. However, the inhibitory effect of DA in this species rests on a single experiment and confirmation of this point would be desirable.

In the human, the evidence available is compatible with the hypothesis that both NE and DA serve as neurotransmitters controlling GH release. The fact that L-dopa administration causes a GH rise is not "per se" proof of a stimulant role of DA, since L-dopa could act to increase NE levels in the hypothalamus or limbic system and this effect would mediate GH release. Quite interestingly in fact, both phentolamine and propranolol interacted with the effect of L-dopa. However, suggestions for a direct stimulation of DA receptors in the release of GH come from the reported stimulant action of apomorphine, the persistence of L-dopa activity following blockade of NE synthesis and the suppressive effects of antidopaminergic drugs. The likelihood of a dopaminergic mechanism in GRF control is also supported by the effectiveness of amphetamine derivatives to stimulate HGH secretion, and action which is not suppressed by the α blocker, thymoxamine (46). It is known, in fact, that some of the central effects of the sympathomimetic amines result by an enhancement of DA receptor activity (47).

The possibility that the stimulatory effect of L-dopa on GH secretion in the human might be due to a final activation of a serotoninergic receptor, for a displacement of the brain indoleamine from vesicular stores (48), is appealing. It appears, however, rather unlikely on considering the very high doses of L-dopa necessary to elicit this effect in the laboratory animal (49) and the limited action of serotonin as GH releaser. Stricly connected with the nature of the transmitter(s) and still unresolved is the problem of its (their) site of action in the brain, a problem, which for reasons of economy cannot be discussed here. Several loci have been proposed at which monoamines might participate in the control of secretion from anterior pituitary (50).

REFERENCES

1. McCann S. M. and Porter J. C. (1969) *Physiol. Rev.* **49,** 240–284.
2. Falck B., Hillarp N. A., Thieme G. and Torp A. (1962) *J. Histochem. Cytochem.* **10,** 348–354.
3. Müller E. E., Saito T., Arimura A. and Schally A. V. (1967) *Endocrinology* **80,** 109–117.
4. Müller E. E., Sawano S., Arimura A. and Schally A. V. (1967) *Endocrinology* **80,** 471–476.
5. Axelrod J. (1965) *Recent Progr. Hormone Res.* **21,** 597–622.
6. Müller E. E., Dal Pra' P. and Pecile A. (1968) *Endocrinology* **83,** 893–896.
7. Müller E. E., Pecile A., Felici M. and Cocchi D. (1970) *Endocrinology* **86,** 1376–1381.
8. Schalch D. S. and Reichlin S. (1966) *Endocrinology* **79,** 275–280.
9. Schalch D. S. and Reichlin S. (1968) In: *Growth Hormone* (Pecile A. and Müller E. E. Eds) pp. 211–225, Excerpta Medica, Amsterdam.
10. Collu R., Fraschini F., Visconti P. and Martini L. (1972) *Endocrinology* **90,** 1231–1237.
11. Kato Y., Dupre' J. and Beck J. C. (1973) *Endocrinology* **93,** 135–146.
12. Andén N. E., Dahlstrom A., Fuxe K. and Larsson K. (1966) *Acta Pharmacol. Toxic.* **24,** 263–274.
13. Corrodi H., Fuxe K., Hamberger B. and Ljungdahl A. (1970) *Europ. J. Pharmac.* **12,** 145–155.
14. Uretsky N. J. and Iversen L. L. (1970) *J. Neurochem.* **17,** 269–273.
15. Spector S., Sjoerdsma A. and Udenfriend S. (1965) *J. Pharm. Exp. Ther.* **147,** 86–95.
16. Glowinski J. and Iversen L. L. (1966) *J. Neurochem.* **13,** 655–669.
17. Müller E. E., Miedico D., Giustina G. and Cocchi D. (1971) *Endocrinology* **88,** 345–350.
18. Sinha Y. N., Selby F. W., Lewis U. J. and Vanderlaan W. P. (1972) *Endocrinology* **91,** 784–792.
19. Machlin L. J., Takahashi Y., Horino M., Hertelendy F., Gordon R. S. and Kipnis D. M. (1968) In: *Growth Hormone* (Pecile A. and Müller E. E., Eds) pp. 292–305. Excerpta Medica, Amsterdam.
20. Hertelendy F., Machlin L. and Kipnis D. M. (1969) *Endocrinology* **84,** 192–199.
21. Davis S. L. and Borger M. L. (1973) *Endocrinology* **92,** 303–309.
22. Lovinger R., Connors M., Boryzcka A., Kaplan S. L. and Grumbach M. M. (1973) *Fifty Fifth Meet. Endocr. Soc.* Chicago.
23. Meyer V. and Knobil E. (1967) *Endocrinology* **80,** 163–171.
24. Werrbach J. H., Gale C. C., Goodner C. J. and Conway M. J. (1970) *Endocrinology* **86,** 77–82.
25. Toivola P. T. K., Gale C. C., Goodner C. J. and Werrback J. H. (1972) *Hormones* **3,** 193–213.
26. Toivola P. T. K. and Gale C. C. (1972) *Endocrinology* **91,** 895–902.
27. Toivola P. T. K. and Gale C. C. (1970) *Neuroendocrinology* **6,** 210–219.
28. Roth J., Glick S. M., Yalow R. S. and Berson S. A. (1963) *Science* **140,** 987–988.
29. Rabinowitz D., Merimee T. J., Nelson J. K., Schultz R. B. and Burgess J. A. (1968) In: *Growth Hormone* (Pecile A. and Müller E. E. eds) pp. 105–115, Excerpta Medica, Amsterdam.
30. Boyd A. E., Lebovitz H. E. and Pfeiffer J. B. (1970) *New Engl. J. Med.* **238,** 1425–1429.
31. Eddy R. L., Lloyd Jones A., Chakmajkan Z. H. and Silverthone M. C. (1971) *Fifty third Meet. Endocr. Soc.* A-210.
32. Kansal P. C., Buse J., Talbert O. R. and Buse M. G. (1972) *J. Clin. Endocr. Metab.* **34,** 99–105.
33. Blackard W. G. and Heidingsfelder S. A. (1968) *J. Clin. Invest.* **47,** 1400–1414.
34. Yawata M. and Fukase M. (1968) *J. Clin. Endocr. Metab.* **28,** 1079–1081.
35. Massara F. and Strumia E. (1970) *J. Endocr.* **47,** 95–100.
36. Massara F. and Camanni F. (1971) *J. Endocr.* **54,** 195–206.
37. Anden N. E., Rubenson A. A., Fuxe K., Hokfelt T. (1967) *J. Pharm. Pharmac.* **19,** 629.
38. Lal S., De La Vega C. E., Sourkes T. L. and Friesen H. G. (1972) *Lancet* **i,** 661.
39. Goldstein M., Anagnoste B., Lauber E. and McKeregan M. R. (1964) *Life Sci.* **3,** 763–767

40. JANSSEN P. A. J., NIEMERGEERS C. J., SCHELLEKENS K. H. L., LENAERTS F. M. VERBRUGGEN F. J. VAN NEUTEN J. M. and SCHAPER W. K. A. (1970) *Europ. J. Pharm.* **11**, 139–154.
41. NEFF N. H. and COSTA E. (1967) In *Antidepressant drugs* pp. 28–34, Excerpta Medica, Amsterdam.
42. KIM S., SHERMAN L., KOLODNY H. D., BENJAMIN F. and SINGH A. (1971) *Clin. Res.* **15**, 718.
43. SHERMAN L., KIM S., BENJAMIN F. and KOLODNY H. D. (1971) *New Engl. J. Med.* **284**, 72–74.
44. FELDMAN J. M. and LEBOVITZ H. E. (1972) In: *Abstracts 4th Int. Congr. Endocr.* p. 35, Excerpta Medica, Amsterdam.
45. IMURA H., NAKAI I., YOSHIMI T. (1973) *J. Clin. Endocr. Metab.* **36**, 204–206.
46. REES L., BUTLER P. W. P., GOSLING C., BESSER G. M. (1970) *Nature, Lond.* **228**, 565–566.
47. CARLSSON A. (1970) In: *Amphetamine and related compounds* (COSTA E. and GARATTINI S., Eds) pp. 289–300 Raven Press, New York.
48. NG K. Y., CHASE T. N., COLBURN R. W. and KOPIN I. J. (1970) *Science* **170**, 76–78.
49. ALGERI S. and CERLETTI C. (1972) In: *Abstracts V° Int. Congr. Pharmac.* p. 4 S. Francisco
50. WURTMAN R. J. (1971) In: *Brain monoamines and endocrine function*, Neur. Res. Progr. Bull. **9**, 214–217.
51. SCHALCH D. S. (1967) *J. Lab. Clin. Med.* **69**, 256–269
52. HEIDINGSFELDER S. A. and BLACKARD W. G. (1968) *Metabolism* **17**, 1019–1024.
53. LUCKE C. and GLICK S. M. (1971) *J. Clin. Endocr. Metab.* **32**, 729–736.
54. BUCKLER J. M. H., BOLD A. M., TABERNER M. and LONDON D. R. (1969) *Brit. Med. J.* **3**, 153–154.