The Effects of Drugs Which Modify Catecholamine Synthesis on Serum Prolactin in Rats with Median Eminence Lesions (37320)

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Considerable evidence has now accumulated to indicate that catecholamines play a role in the regulation of prolactin secretion in the rat. The injection of dopamine into the third ventricle of normal male (1) or lactating female rats (2) results in a decrease in plasma prolactin. This has been thought to be due to a release of prolactin-inhibiting factor (PIF) since incubation of dopamine with hypothalami *in vitro* led to an increase of PIF activity in the medium (2) and portal blood from dopamine treated animals contained increased PIF activity (1). Infusion of dopamine into a cannulated portal vessel had no effect on prolactin release, suggesting that dopamine was without action at the pituitary level (1); however, incubation of pituitaries with dopamine *in vitro* inhibited prolactin release, suggesting direct action on the gland (2–4).

Further evidence for the participation of dopamine as an inhibitory transmitter in the regulation of prolactin release was provided by experiments in which drugs which augment or inhibit catecholamine synthesis were injected into rats. Drugs such as dihydroxyphenylalanine (L-DOPA), which lead to an augmentation of central catecholamine synthesis, caused a fall in plasma prolactin, whereas drugs such as alpha methyl paratyrosine, which inhibit catecholamine synthesis, led to dramatic elevations in plasma prolactin (5). Using drugs which specifically augment or inhibit dopamine synthesis, evidence was provided for an inhibitory role of dopamine in controlling prolactin release (5).

If dopamine acts at the CNS to inhibit prolactin release, one would expect no effect of the drug in animals in which CNS control of prolactin release has been interrupted. In the present experiments, CNS control was interrupted by means of median eminence lesions. When L-DOPA was injected to augment dopamine synthesis, a dramatic decline in plasma prolactin occurred in the animals suggesting that elevated levels of dopamine can act directly at the pituitary level to inhibit prolactin release by the gland.

Methods. Rats of the Holtzman strain (Holtzman Laboratories, Madison, WI) were housed in an air-conditioned animal room with controlled lighting (lights on 5 AM–7 PM), fed laboratory chow and given free access to water. The animals were castrated and 2–3 weeks later all animals received median eminence (ME) lesions produced by passing a direct anodal current of 3 mA for 15 sec as described previously (6, 7). One week after the lesions had been placed, the animals were used for experiments.

At time 0, the animals were anesthetized with ether and a blood sample of 1.0 ml was quickly obtained. The various drugs were injected ip at time 0 or later on and additional blood samples were obtained in the same manner at varying times after initiation of the experiment. After centrifugation of the samples, serum prolactin was determined using the kits supplied by NIAMD and the results were expressed in terms of the RP-1-rat pituitary prolactin reference standard[4].

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The following drugs were used: DL-alpha-methyltyrosine methylester hydrochloride (α-MT) (Hassle, Sweden, generously provided by Dr. Hans Corrodi and from Regis Chemical) and sodium diethyldithiocarbamate (DDC) (Fisher) were used to block the synthesis of catecholamines (8, 9) and of norepinephrine (10), respectively. L-3,4-dihydroxyphenylalanine (L-DOPA) (Regis Chemical) and DL-threo-3,4-hydroxyphenylserine (DL-DOPS) (Regis Chemical) were used as precursors of dopamine and norepinephrine, respectively (11). The doses of the drugs are given in the results.

At the termination of the experiment the brains were prepared for histological study to determine the extent of the median eminence lesions as previously described (6, 7).

Differences between means of groups were calculated by the paired t test.

Results. The effect of ME lesions on serum prolactin. In accord with previous results (6, 7), ME lesions uniformly resulted in elevated serum prolactin levels in castrate rats of either sex (Tables I–III).

The effect of L-DOPA in rats with ME lesions. When L-DOPA was injected to augment catecholamine synthesis, it evoked a dramatic decrease in the elevated prolactin levels in the rats with median eminence lesions (Table I). The decline came on within 30 min of injection and persisted for at least 90 min.

The effect of α-methyltyrosine (α-MT) on plasma prolactin in rats with ME lesions. Since α-MT had produced dramatic elevations in serum prolactin in animals with intact brains (5), it was injected into the animals with ME lesions. In contrast to its effect in the normal animal, α-MT had little if any effect in animals with lesions (Table II). There was a small but significant elevation in plasma prolactin ($p < 0.05$) in one of four experiments. There was no significant effect in the other three experiments, and, in fact, in one of the three plasma prolactin actually declined somewhat. When L-DOPA was injected 1 hr after the injection of α-MT, it was effective in lowering plasma prolactin 1 hr later.

Lack of effect of drugs which alter norepinephrine synthesis on serum prolactin in rats with ME lesions. In intact animals DL-DOPS, an agent which selectively stimulates norepinephrine synthesis, elevated plasma prolactin (5). In a small group of animals with lesions (Table III), it had no effect. When DDC was injected to block conversion of dopamine to norepinephrine, it also had no effect in the animals with lesions. Furthermore, L-DOPA was still effective in lowering plasma prolactin in these animals, even though its conversion to norepinephrine had presumably been blocked.

Completeness of lesions. The lesions in the animals appeared to be complete as evidenced by the absence of normal tissue in the median

TABLE I. Effect of L-DOPA (100 mg/kg) on Serum Prolactin (ng/ml) in Rats with ME Lesions.

Type of rat	Expt. no.	Treatment	Time of sampling (min) 0	30	60
Castrate males	1	Saline	98 ± 16 (10)[a]	87 ± 17 (6)	113 ± 17 (6)[b]
		L-DOPA		10.0 ± 2.1 (4)**	5.8 ± 0.4 (4)[b]***
Castrate males	2	Saline	127 ± 13 (5)	118 ± 14 (5)	123 ± 17 (5)
		L-DOPA	142 ± 10 (5)	8 ± 2 (5)***	6 ± .3 (5)***
Castrate females	3	Saline	179 ± 14 (5)	197 ± 21 (5)	215 ± 32 (5)
		L-DOPA	216 ± 28 (4)	92 ± 23 (4)**	51 ± 7 (4)***

[a] Mean ± SEM (no. of rats).
[b] Taken at 90 min.
* $p < 0.05$ vs 0 time control.
** $p < 0.01$.
*** $p < 0.001$.

TABLE II. Effects of α-Methyltyrosine (200 mg/kg) and L-DOPA (100 mg/kg) on Serum Prolactin (ng/ml) in Rats with ME Lesions.

Type of rat	Expt. no.	Treatment	Time of sampling (hr) 0	1	2	4
Castrate males	1	α-MT—0 time	111 ± 7 (5)	93 ± 13 (5)	108 ± 11 (5)	103 ± 12 (5)
		Saline—0 time	113 ± 5 (4)	125 ± 17 (4)	99 ± 13 (4)	108 ± 11 (4)
Castrate females	2	α-MT—0 time L-DOPA—1 hr	106 ± 8 (5)	130 ± 10 (5)*	54 ± 9 (5)***	
		α-MT—0 time Saline—1 hr	126 ± 12 (5)	155 ± 21 (5)	144 ± 10 (5)	
Castrate females	3	α-MT—0 time L-DOPA—1 hr	58 ± 4 (10)	71 ± 6 (10)	27 ± 4 (5)**	

* $p < 0.05$ vs 0 time control.
** $p < 0.01$ vs 0 time control.
*** $p < 0.01$ vs 1 hr.

eminence-arcuate region. The completeness of lesions is also suggested by the elevated levels of prolactin which were found in the animals and are characteristic of animals with ME lesions (6, 7). Furthermore, plasma gonadotropins were measured in the castrate animals and were found to be low indicative of the interruption of CNS control of gonadotropin release as well.

Discussion. The dramatic decline in the elevated levels of prolactin induced by L-DOPA in the animals with lesions was surprising and suggested at face value that an elevation in catecholamine synthesis can lead to enhanced release of catecholamines which inhibit prolactin by a direct action at the pituitary level. That the catecholamine responsible is probably dopamine is suggested by the experiments with DDC which would block the conversion of dopamine into norepinephrine. In these animals, L-DOPA was equally effective to lower prolactin as in the controls. In a preliminary experiment, DL-DOPS which was given to augment norepinephrine synthesis selectively failed to influence prolactin. This suggests that any norepinephrine which reaches the pituitary in rats with ME lesions is insufficient to influence prolactin release. It is still possible that norepinephrine might stimulate prolactin release by an action on the hypothalamus (5).

The histological evidence suggests that the lesions in the median eminence in this study were complete. This interpretation is strengthened by the fact that plasma prolactin levels were elevated as they are when pituitaries are removed from inhibitory CNS control either by hypothalamic lesions or by grafting the gland to a distant site (6, 7, 12).

TABLE III. Lack of Effect of DL-DOPS (200 mg/kg) or DDC (500 mg/kg) on Serum Prolactin (ng/ml) in Rats with ME Lesions.

Type of rat	Expt. no.	Treatment	Time of sampling (hr) 0	1	2	4
Castrate males	1	DL-DOPS	70 ± 20 (4)	74 ± 26 (5)		
		Saline		66 ± 25 (4)		
Castrate males	2	DDC—0 hr / L-DOPA—1 hr	114 ± 5 (5)	105 ± 12 (5)	17 ± 4 (5)***	7 ± 0.5 (5)***
		Saline	113 ± 6 (4)	125 ± 17 (4)	99 ± 13 (4)	108 ± 11 (4)

*** $p < 0.001$ vs 1 hr.

The low levels of gonadotropins which were found are characteristic of animals with complete ME lesions (6, 7). The negligible response to blockade of catecholamine synthesis by α-methyltyrosine is also consistent with the view that these lesions were complete. This drug produced a dramatic elevation in plasma prolactin in intact animals (5), but had little if any effect in the animals with lesions.

Since α-MT did not lead to a release of prolactin in animals with lesions, it would appear that levels of catecholamines either circulating or in the pituitary gland have no influence on plasma prolactin in these rats. It is still possible that catecholamines released into the portal vessels could lower prolactin release in normal animals by a direct action on the gland.

Thus, the present results suggest that when L-DOPA is given to augment catecholamine synthesis, high levels of catecholamines in particular dopamine, may reach the pituitary either via the systemic circulation or more probably via local release in the gland to inhibit prolactin release directly. In support of the local release hypothesis is the finding that administered L-DOPA is decarboxylated to dopamine and stored in the periodic acid Schiff-positive cells of the rat adenohypophysis for many hours (13). The normal hypothalamic restraining influence on prolactin release appears to be mediated largely by central catecholamines, in particular dopamine, which act through release of PIF (1, 2, 5) or by inhibiting the release of PRF (14) or by both processes.

Summary. 1. In animals with median eminence lesions and already elevated serum prolactin, L-DOPA produced a dramatic decline in serum prolactin within 30 min of its injection. L-DOPA was still effective if its conversion to norepinephrine was blocked by DDC.

2. α-methyl para-tyrosine to inhibit catecholamine synthesis produced little if any effect on the elevated levels of prolactin which suggests that the lesions were complete and that catecholamines exert no inhibitory effect on prolactin release at the pituitary level under these conditions.

3. Drugs which either stimulated or inhibited norepinephrine synthesis had no effect on serum prolactin in animals with lesions.

4. The results suggest that the inhibitory CNS control over prolactin release is mediated by hypothalamic dopaminergic synapses, but also suggest that pharmacologically elevated levels of catecholamines, in particular dopamine, can inhibit prolactin release at the pituitary level.

1. Kamberi, I. A., Mical, R., and Porter, J. C., Endocrinology **88**, 1012 (1971).

2. Kuhn, E., Krulich, L., Quijada, M., Illner, P., Kalra, P. S., and McCann, S. M., Prog. 52nd Mtg. Endocrine Soc. 126 (1970).

3. MacLeod, R. M., Endocrinology **85**, 916 (1969).

4. Birge, C. A., Jacobs, L. S., Hameo, C. T., and Daughaday, W. H., Endocrinology **86**, 120 (1970).

5. Donoso, A. O., Bishop, W., Fawcett, C. P., Krulich, L., and McCann, S. M., Endocrinology **89**, 774 (1971).

6. Bishop, W., Krulich, L., Fawcett, C. P., and McCann, S. M., Proc. Soc. Exp. Biol. Med. **136**, 925 (1971).

7. Bishop, W., Fawcett, C. P., Krulich, L., and McCann, S. M., Endocrinology **91**, 643 (1972).

8. Spector, S., Sjoerdsma, A., and Undenfriend, S., J. Pharmacol. **147**, 86 (1965).

9. Corrodi, H., and Hanson, L. C. F., Psychopharmacologia **10**, 116 (1966).

10. Carlsson, A., Fuxe, K., Hökfelt, T., and Lindqvist, M., J. Pharm. Pharmacol. **18**, 60 (1966).

11. Creveling, C. R., Daly, J., Tokuyama, T., and Witkop, B., Biochem. Pharmacol. **17**, 65 (1968).

12. Meites, J., Lu, K. H., Wuttke, W., Welsch, C. W., Nagasawa, H., and Quadri, S. K., Rec. Prog. Hormone Res. **28**, 471 (1972).

13. Dahlström, A., and Fuxe, K., Acta Endocrinol. (Kbh.) **51**, 301 (1966).

14. Ajika, K., Krulich, L., and McCann, S. M., Proc. Soc. Exp. Biol. Med. **141**, 203 (1972).

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