Inhibition by L-Dopa and Monoamine Oxidase Inhibitors of Pituitary Prolactin Release; Stimulation by Methyldopa and *d*-Amphetamine (35604)

KUEW-HSIUNG LU AND J. MEITES[1]

Department of Physiology, Michigan State University, East Lansing, Michigan 48823

Numerous studies have indicated that hypothalamic catecholamines (CA) influence secretion of several anterior pituitary (AP) hormones, including prolactin. An adrenergic tonus is believed to inhibit prolactin release, and a reduction in hypothalamic CA's to promote prolactin release (1, 2). Recently we reported that a single intracarotid injection of dopamine, epinephrine, but not norepinephrine, evoked small but significant decreases in AP prolactin levels but no significant changes in serum prolactin levels (3). On the other hand, a single injection of reserpine, chlorpromazine, α-methyl-*para*-tyrosine or α-methyl-*meta*-tyrosine, all known to depress hypothalamic CA's (2, 3), produced a rapid increase in serum prolactin concentration and, with the exception of α-methyl-*meta*-tyrosine, a decrease in AP prolactin concentration.

Inasmuch as dopamine, epinephrine, and norepinephrine do not readily pass through the blood–brain barrier (4, 5), the present study was undertaken to determine whether other drugs known to enter the brain and increase CA levels could depress serum prolactin values. The drugs used included L-dopa, the immediate precursor of dopamine; 3 monoamine oxidate inhibitors (pargyline, Lilly-15641, and iproniazid) that depress normal metabolism of epinephrine and norepinephrine and therefore increase brain catecholamine levels; methyldopa, which inhibits dopa decarboxylase and reduces synthesis of dopamine and norepinephrine; and *d*-amphetamine, a sympatheticomimetic drug.

Materials and Methods. Animals. Mature 4- to 5-month-old female Sprague-Dawley rats (Spartan Research Animals, Haslett, Mich.), weighing 225–255 g each, were used in all experiments. The rats were housed in a temperature-controlled (25±1°) and artificially illuminated (lights on at 5:00 a.m., off at 7:00 p.m.) animal room, and maintained on Wayne Lab Blox pellets and water *ad libitum*. Two complete estrous cycles of 4 to 5 days duration were followed on all female rats before they were injected with one of the drugs at 10 a.m. on the day of proestrus. Since the main objective of these experiments was to determine whether some of the drugs could depress serum prolactin levels, proestrous rats with relatively high serum prolactin levels were used. The experiments were terminated 2 hr (12 a.m.) after each injection.

Drugs. The rats were injected intraperitoneally once with one of the following drugs:[2] L-dopa, methyldopa, pargyline hydrochloride, iproniazid phosphate, *d*-amphetamine sulfate, and Lilly-15641. All drugs except L-dopa and methyldopa were dissolved directly in neutral phosphate buffer saline. The L-dopa and methyldopa were each dissolved in a warm solution of 0.5 *N* HCl to which 0.5 *N* NaOH was added to bring the pH to 2.8. Two control groups were injected once with saline or pH 2.8 solution. The doses of drugs used represents the total amount given per rat, and similar doses were shown by us and other investigators to be effective in rats (1, 8, 9).

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TABLE I. Effects of a Single Intraperitoneal Injection of Drugs on Serum and Pituitary Prolactin Concentrations.

Treatment (7 rats/group) per rat	Serum prolactin (ng/ml)				AP prolactin conc (μg/mg of AP)
	Pre-treatment	30 min	1 hr	2 hr	
pH 2.8 solution, 0.3 ml (controls)	158.6 ± 16.4[a]	143.6 ± 12.0	146.4 ± 10.3	130.1 ± 9.7	10.34 ± 0.19
L-dopa, 12 mg	174.1 ± 12.7	85.1 ± 3.2[c]	90.1 ± 8.8[c]	66.7 ± 4.1[c]	12.08 ± 0.79[b]
Methyldopa, 80 mg	204.3 ± 21.8	955.3 ± 85.3[c]	1319.1 ±171.5[c]	984.1 ±188.1[c]	8.19 ± 0.28[c]
Saline, 0.5 ml (controls)	169.6 ± 10.1	193.1 ± 26.7	192.9 ± 24.6	181.9 ± 11.6	9.32 ± 0.29
Pargyline, 15 mg	187.4 ± 15.5	112.7 ± 7.0[c]	107.1 ± 10.9[c]	100.0 ± 11.1[c]	9.35 ± 0.37
Pargyline, 15 mg + L-dopa, 12 mg	149.6 ± 13.3	66.7 ± 3.0[c]	63.7 ± 6.0[c]	70.1 ± 5.4[c]	10.63 ± 0.11
Lilly-15641, 8 mg	208.0 ± 28.9	208.6 ± 32.9	173.1 ± 25.2	126.0 ± 21.7[b]	11.63 ± 0.44[c]
Iproniazid, 40 mg	195.6 ± 25.5	182.6 ± 40.0	168.7 ± 38.1	89.4 ± 13.8[c]	8.89 ± 0.37
d-Amphetamine, 1.2 mg	142.9 ± 6.7	568.3 ±126.1[c]	1056.1 ±211.4[c]	956.1 ±245.1[c]	5.88 ± 0.32[c]

[a] Mean ± standard error of mean.

[b] Significantly different from controls: $p < 0.05$; [c] $p < 0.01$.

Collection of sera and AP's for assay. Individual blood samples (0.6 or 0.7 ml) were collected by cardiac puncture under light ether anesthesia at 0, 0.5, 1, and 2 hr following each treatment. This method of blood collection was previously shown not to alter normal serum prolactin values in rats (6). A pretreatment blood sample was taken from each animal prior to drug injection for comparison with subsequent blood samples. The animals were decapitated by guillotine after the last blood samples were collected. The AP's were removed immediately, weighed on a Mettler balance, and homogenized in neutral phosphate buffer saline with a ultrasonic cell disruptor. Blood samples were first stirred gently and then placed in a refrigerator at 4° for 24 hr to separate the serum. The sera and AP homogenates were kept in a freezer at —20° until assayed for prolactin.

Prolactin assay. Prolactin activity in individual serum samples and AP homogenates were assayed by radioimmunoassay (7). Each sample was assayed at two dilutions, averaged, and expressed in terms of a purified rat prolactin reference standard (NIAMD-Rat-Prolactin-RP-1) obtained from NIH, Rat Pituitary Hormone Program, Bethesda, Md. Sample means and standard error of means were calculated for each experimental group. Student's *"t"* test was used to determine significance of differences in serum prolactin values between pretreatment and posttreatment samples, and also for differences in AP prolactin concentrations between groups.

Results. Table I shows that a single intraperitoneal injection of saline or pH 2.8 solution (controls) had no effect on subsequent serum prolactin levels. Administration of L-dopa reduced serum prolactin about 50% by 30 min or 1 hr after injection as compared to pretreatment values. By 2 hr after injection serum prolactin values were reduced about 60% and AP prolactin concentration was significantly increased. Pargyline produced smaller reductions in serum prolactin and no significant change in AP prolactin concentration. When both L-dopa and pargyline were given together, there were greater decreases in serum prolactin values than when either drug was given alone. The combination produced no significant change in AP prolactin concentration. Lilly-15641 or iproniazid produced no effect on serum prolactin by 30 min or 1 hr after injection, but elicited about a 40–50% reduction by 2 hr after injection. Lilly-15641 but not iproniazid significantly increased AP concentration of prolactin.

A single injection of amphetamine increased serum prolactin over pretreatment

values about 4-fold by 30 min, about 7-fold by 1 hr and about 6-fold by 2 hr after injection. This drug reduced AP prolactin concentration by about 44%. An injection of methyldopa increased serum prolactin over pretreatment levels about 5-fold by 30 min, about 6-fold by 1 hr, and about 5-fold by 2 hr after injection. This drug significantly reduced AP prolactin concentration.

Discussion. These results demonstrate that a single injection of L-dopa, the precursor of dopamine, produced a rapid decrease in serum prolactin and an increase in AP prolactin concentration. The ability of L-dopa to raise brain CA levels (2, 4, 5) is believed to be responsible for the decrease in prolactin release. Rapid reductions in serum prolactin were also observed shortly after L-dopa injection by Wedig and Gay (8) and Schneider and Midgley (9), but the latter noted an increase in serum prolactin by 26 to 100 min after injection. We did not observe any increase in serum prolactin levels even by 2 hr after injecting L-dopa, and have recently confirmed this in another experiment with several doses of L-dopa (unpublished).

The monoamine oxidase inhibitors, pargyline, Lilly-15641 and, iproniazid, all reduced serum prolactin levels significantly, presumably by interfering with the metabolism of CA's (5) and thereby increasing their concentration in the hypothalamus. Pargyline was the most effective of the 3 monoamine oxidase inhibitors used for decreasing serum prolactin concentration, and when given together with L-dopa, the reduction in serum prolactin was greater than when either drug was given alone, presumably because synthesis of CA was enhanced and metabolism of CA was reduced. We previously reported that iproniazid inhibited mammary growth and lactation (10), and depressed mammary tumor growth in rats (11), although no significant change in serum prolactin was found. Since blood samples were collected from these animals about 24 hr after the last injection of iproniazid, it is possible that any decrease which may have occurred earlier was missed.

The large increase in serum prolactin and significant fall in pituitary prolactin produced by injection of methyldopa is probably due to a reduction in brain CA's, since this drug interferes with synthesis of dopamine and norepinephrine (1, 4). The action of amphetamine is more difficult to understand, although it has been observed that amphetamine induces a brief liberation of norepinephrine from adrenergic terminals and that it may inhibit monoamine oxidase activity (4, 5). Amphetamine also blocks dopamine reuptake by nerve endings (Fuller, personal communication), which would block synthesis of norepinephrine. Further work will be necessary to elucidate the stimulating action of amphetamine on prolactin release.

In general, the results reported here are in agreement with the concept that an adrenergic tonus in the hypothalamus inhibits prolactin release (1, 2). Recent work by Kamberi *et al.* (12) has shown that an injection of dopamine into the 3rd ventricle of rats increases PIF activity in the hypothalamo-pituitary portal blood and decreases serum concentration of prolactin. The present study demonstrates that several drugs known to enhance brain CA activity produce a decrease in AP prolactin release. Drugs that reduce hypothalamic CA activity, including methyldopa, reserpine, and chlorpromazine, increase prolactin release. Possible direct effects of these drugs on the AP can not be excluded, although CA have been reported to produce equivocal effects on AP prolactin release *in vitro* (13).

Summary. A single intraperitoneal injection of L-dopa, the precursor of dopamine, into female rats significantly reduced serum prolactin concentration at 30 min, 1 hr, and 2 hr after injection compared to pretreatment levels or control rats not given this drug. A single injection of each of 3 monoamine oxidase inhibitors, pargyline, iproniazid or Lilly-15641, also significantly decreased serum prolactin below pretreatment values. Injection of L-dopa and pargyline together was more effective than either alone in lowering serum prolactin. Each of the above drugs is believed to reduce pituitary prolactin release because it increases catecholamine activity in the hypothalamus. By contrast, a single injection of methyldopa, which inhibits

catecholamine synthesis, increased serum prolactin many fold over pretreatment levels. A single injection of *d*-amphetamine, a sympatheticomimetic drug, also greatly increased serum prolactin concentration.

Addendum

The hypothalami of the rats treated with saline (controls), iproniazid, pargyline, L-dopa or pargyline and L-dopa together, were assayed for PIF activity by our standard *in vitro* procedure. All drugs increased hypothalamic PIF activity and the combination of pargyline and L-dopa was more effective than either alone. These observations suggest that the increase in hypothalamic catecholamines produced by these drugs results in increased hypothalamic PIF activity, and this in turn depresses prolactin release by the pituitary.

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