*Pharmacology Biochemistry & Behavior*, Vol. 15, pp. 933–936, 1981. Printed in the U.S.A.

# Repeated Administration of Pergolide to Rats Attenuates the Acute Elevation of Serum Corticosterone by Pergolide

RAY W. FULLER AND HAROLD D. SNODDY

*The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285*

Received 30 July 1981

FULLER, R. W. AND H. D. SNODDY. *Repeated administration of pergolide to rats attenuates the acute elevation of serum corticosterone by pergolide.* PHARMAC. BIOCHEM. BEHAV. **15**(6) 933–936, 1981.—A single injection (0.3 mg/kg IP) of pergolide mesylate, a dopamine agonist, increased serum corticosterone concentration several-fold in rats. This increase did not occur or was greatly diminished in rats that had received four previous daily injections of pergolide. The non-responsiveness to pergolide persisted at the second day after the last of 4 daily injections of pergolide, but by the third and fourth days the rats were again responsive to pergolide in terms of serum corticosterone elevation. The lowering of dopamine metabolites (3,4-dihydroxyphenylacetic acid and homovanillic acid) by pergolide occurred in pergolide-pretreated rats to the same extent as in control rats. Serum corticosterone concentration in pergolide-pretreated rats was elevated normally by a serotonin agonist, quipazine. Since the pituitary-adrenocortical system can be activated in pergolide-pretreated rats (indicated by the normal response to quipazine), the insensitivity to pergolide may be due to decreased responsiveness of central dopamine receptors mediating corticosterone elevation by pergolide.

Pergolide  Serum corticosterone  Quipazine  Dopamine metabolites  Dopamine receptors

RECENTLY we reported that pergolide mesylate, a dopamine agonist [2], caused a dose-dependent increase in serum corticosterone concentration in rats [3]. The effect was rapid, corticosterone concentration being maximally increased at 30–60 minutes and returning to control levels by 4 hours. Several lines of evidence suggested the effect was a consequence of activation of central dopamine receptors by pergolide. The increase was blocked by pretreatment with spiperone or haloperidol, antagonists of central dopamine receptors, but not by domperidone, a compound that blocks dopamine receptors in the periphery but not in brain *in vivo*. Other direct and indirect dopamine agonists also elevated serum corticosterone concentration in rats, namely lergotrile, apomorphine, N,N-dipropyl-2-aminotetralin, amfonelic acid and L-dopa, but a close structural analog of pergolide relatively inactive as a dopamine agonist did not.

In this paper, we are reporting that a dose of pergolide initially producing maximum elevation of serum corticosterone has little or no effect in rats that have received 4 previous daily injections of pergolide. In contrast, a biochemical effect—depression of dopamine metabolites in brain—occurs unchanged in pergolide-pretreated rats.

## METHOD

Male Wistar rats from Harlan Industries, Cumberland, IN, were acclimated to the animal room for at least one week prior to an experiment. The rats were kept in hanging wire cages in groups of five in a 24°C room with food and water freely available and lights on from 0700–1900 hours. At the time an experiment was started, the rats weighed approximately 200 g each. Pergolide mesylate and quipazine maleate were synthesized in the Lilly Research Laboratories; we are grateful to Drs. E. C. Kornfeld and B. B. Molloy, respectively, for these compounds. Drugs were injected IP, and rats were killed by decapitation between 9 a.m. and noon, when serum corticosterone concentration is low in the normal diurnal rhythm. Trunk blood was collected and allowed to clot. Serum was obtained by centrifugation and assayed immediately or stored frozen at −15° prior to assay.

Corticosterone concentration was assayed spectrofluorometrically by the method of Solem and Brinck-Johnsen [8]. Dopamine metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid, were measured in whole brain by liquid chromatography with electrochemical detection [7]. All data are expressed as mean values ± standard errors for five rats per group. Comparisons between groups were made by Student's *t*-test.

## RESULTS

Table 1 shows the effect of the first and the fifth daily injections of pergolide. The 0.3 mg/kg dose was chosen because previous studies [3] had shown it to produce a maximal increase in serum corticosterone within 30–60 min after a single injection of pergolide. At this dose, pergolide caused a 7-fold increase in serum corticosterone concentration acutely after the first injection. The last of five daily injections, in contrast, caused only a small effect.

Figure 1 shows a dose-response curve for pergolide in naive rats and in rats that had received four previous daily

TABLE 1

EFFECT OF 4 DAILY INJECTIONS OF PERGOLIDE ON THE ACUTE ELEVATION OF SERUM CORTICOSTERONE BY PERGOLIDE

| Last injection | Serum corticosterone, μg/100 ml: Daily vehicle injections | Daily pergolide injections | Difference |
|---|---|---|---|
| Vehicle | 7.08 ± 0.24 | 6.10 ± 0.55 | n.s. |
| Pergolide mesylate 0.3 mg/kg | 49.78 ± 3.17* | 13.18 ± 2.23* | $p<0.001$ |

*Significant difference from vehicle-treated group in same column ($p<0.05$).

Serum corticosterone levels are shown for rats that received four daily injections of vehicle (left column) or of pergolide (right column) prior to the fifth day, when they were killed 1 hr after receiving an injection of vehicle (top line) or of pergolide (bottom line). The dose of pergolide mesylate was 0.3 mg/kg in all cases, and all injections were IP.

injections of pergolide. In naive rats, doses of 0.1 and 0.3 mg/kg caused a dose-related increase in corticosterone concentration, whereas the 0.03 mg/kg dose was without effect. In rats previously treated with pergolide, neither the 0.1 nor the 0.3 mg/kg doses had any effect on corticosterone concentration. A 1 mg/kg dose caused an apparent effect in some rats, but the response was so variable that a statistically significant difference from control did not occur in this group.

Table 2 shows that rats pretreated with 4 daily injections of pergolide responded normally to quipazine. Quipazine caused a dose-related increase in serum corticosterone concentration in control rats and in pergolide-pretreated rats, the differences between the two groups not being significant at any dose of quipazine.

Table 3 shows the ability of pergolide to decrease brain concentrations of two dopamine metabolites, DOPAC (3,4-

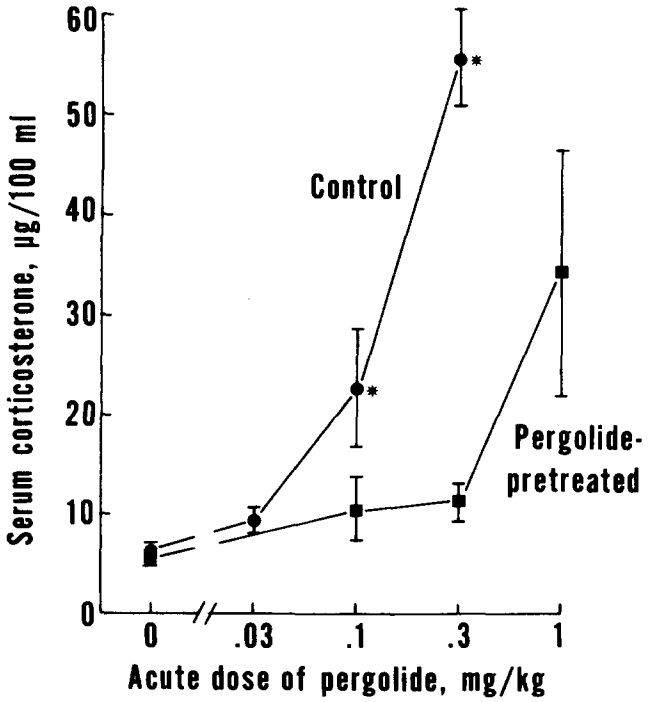


FIG. 1. Shift in dose-response curve for corticosterone elevation by pergolide as a result of daily pretreatment with pergolide. Pergolide mesylate (0.3 mg/kg, IP) was injected daily for 4 days into the pergolide-pretreated group. On day 5, graded doses of pergolide mesylate as indicated were injected 1 hr before rats were killed. Vehicle was injected into the zero dose group. Asterisks indicate significant differences from the corresponding zero dose group.

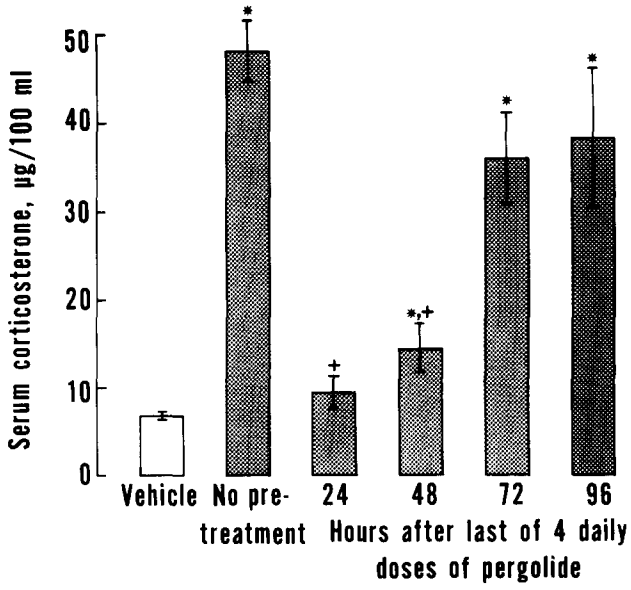


FIG. 2. Duration of the nonresponsiveness to pergolide after the last of 4 daily injections of pergolide. Shaded bars show groups treated with pergolide mesylate (0.3 mg/kg IP) 1 hr before they were killed. Some groups has been pretreated with 4 daily injections of this dose of pergolide, the fourth dose being 24, 48, 72 or 96 hours prior to the injection of pergolide for determination of corticosterone elevation. The asterisk (*) indicates significant elevation of corticosterone ($p<0.05$), and the symbol (+) indicates a significant difference from the group receiving pergolide with no pretreatment ($p<0.05$).

TABLE 2

DOSE-DEPENDENT ELEVATION OF SERUM CORTICOSTERONE BY QUIPAZINE MALEATE IN CONTROL RATS AND IN RATS PRETREATED WITH DAILY DOSES OF PERGOLIDE

| Dose of quipazine maleate (mg/kg IP) | Serum corticosterone, μg/100 ml | |
|---|---|---|
| | Control | Pergolide-pretreated |
| 0 | 4.08 ± 0.25 | 5.04 ± 0.69 |
| 2 | 35.28 ± 8.67* | 32.00 ± 6.09* |
| 4 | 38.30 ± 8.58* | 37.53 ± 9.99* |
| 8 | 46.12 ± 1.52* | 50.65 ± 6.64* |

*Significant difference from zero dose group in same column ($p<0.05$).

Serum corticosterone was measured 1 hr after the injection of quipazine maleate (at the dose indicated) or vehicle. Some rats had received pergolide mesylate (0.3 mg/kg IP) 24, 48, 72 and 96 hrs before the injection of quipazine.

TABLE 3

REDUCTION IN DOPAMINE METABOLITE CONCENTRATION ONE HOUR AFTER PERGOLIDE INJECTION

| Dose of pergolide mesylate (mg/kg IP) | Metabolite Concentration, ng/g | |
|---|---|---|
| | DOPAC | HVA |
| Control rats | | |
| 0 | 76 ± 3 | 80 ± 2 |
| 0.1 | 64 ± 3* | 60 ± 2* |
| 0.3 | 60 ± 2* | 50 ± 2* |
| Pergolide-pretreated rats | | |
| 0 | 88 ± 2 | 90 ± 5 |
| 0.1 | 61 ± 4* | 58 ± 0.1* |
| 0.3 | 63 ± 2* | 54 ± 2* |

*Significant lowering ($p<0.05$).

Metabolite concentrations were measured 1 hr after the injection of pergolide mesylate. Some rats had been pretreated with pergolide mesylate (0.3 mg/kg, IP, once daily on the 4 preceding days).

dihydroxyphenylacetic acid) and HVA (homovanillic acid). Both 0.1 and 0.3 mg/kg doses of pergolide produced similar, statistically significant decreases in the two metabolites in control rats and in rats that had received four previous daily injections of pergolide. The degree of lowering of these metabolites was not diminished in the pergolide-pretreated rats.

Figure 2 shows the effect of pergolide given 1, 2, 3 or 4 days after the last of 4 daily injections of pergolide. As the previous experiments had shown, pergolide given 1 day after the last of 4 daily injections failed to elevate serum corticosterone concentration. This diminished responsiveness to pergolide persisted at the second day, but by days 3 and 4 a significant increase in serum corticosterone was produced by pergolide, and the magnitude of the increase was not significantly less than in naive rats.

## DISCUSSION

The acute elevation of serum corticosterone concentration by pergolide shown in these experiments is in confirmation of our earlier results [3]. Previous evidence that this increase is mediated by stimulation of central dopamine receptors is summarized in the introduction. The new finding in this paper is that the ability of pergolide to elevate serum corticosterone concentration acutely in rats is essentially lost after daily injections of pergolide for 4 days. Rats remain unresponsive to pergolide for at least 2 days after the last of 4 daily injections but begin to respond again to pergolide by 3 or 4 days after the fourth daily dose.

Repeated injection of pergolide does not render the pituitary-adrenocortical system unresponsive to activation, since quipazine elevated serum corticosterone normally in pergolide-pretreated rats. Quipazine elevates serum corticosterone by activation of serotonin receptors [1,4]. Apparently dopamine receptors that mediate the effect of pergolide become unresponsive or less responsive in rats treated chronically with pergolide.

Pergolide had earlier been reported to lower dopamine turnover, one manifestation of which is a decrease in the concentration of dopamine metabolites, DOPAC and HVA [2]. This effect of pergolide was not altered in rats previously given 4 daily injections of pergolide. This effect also is thought to be mediated by dopamine receptors, possibly presynaptic autoreceptors on dopamine neurons, and our data do not indicate that these receptors become subsensitive after the 4 daily injections of pergolide.

Lew *et al.* [6] have reported that daily injections of pergolide (0.2 mg/kg IP) for two weeks in rats leads to diminished specific binding of tritiated spiperone to striatal membrane sites. Since spiperone is thought to bind to dopamine receptors in striatum, their findings suggest that adaptive changes in dopamine receptors had occurred. Fuxe *et al.* [5] have shown a reduced density of binding sites for tritiated N-propylnorapomorphine, another radioligand for striatal dopamine receptors, after administration of pergolide by osmotic minipump at a dose of 0.5 mg/kg/day for two weeks. Although there is no evidence that the dopamine receptors mediating the serum corticosterone elevation by pergolide are located in the striatum, the findings of Lew *et al.* [6] and of Fuxe *et al.* [5] suggest that similar adaptive changes may occur in the dopamine receptors that mediate serum corticosterone elevation by pergolide.

## REFERENCES


1. Fuller, R. W. Serotonergic stimulation of pituitary-adrenocortical function in rats. *Neuroendocrinology* **32:** 118–127, 1981.
2. Fuller, R. W., J. A. Clemens, E. C. Kornfeld, H. D. Snoddy, E. B. Smalstig and N. J. Bach. Effects of (8β-8-[(methylthio)-methyl]-6-propylergoline on dopaminergic function and brain dopamine turnover in rats. *Life Sci.* **24:** 375–382, 1979.
3. Fuller, R. W. and H. D. Snoddy. Elevation of serum corticosterone in rats by pergolide and other dopamine agonists. *Endocrinology* **109:** 1026–1032, 1981.
4. Fuller, R. W., H. D. Snoddy and J. A. Clemens. The effect of quipazine, a serotonin receptor agonist, on serum corticosterone concentration in rats. *Encocr. Res. Communs.* **5:** 161–171, 1978.
5. Fuxe, K., L. F. Agnati, C. Kohler, D. Kuonen, S.-O. Ogren, K. Andersson and T. Hokfelt. Characterization of normal and supersensitive dopamine receptors: effects of ergot drugs and neuropeptides. *J. Neural Transm.* **51:** 3–37, 1981.
6. Lew, J. Y., S. Nakamura, A. F. Battista and M. Goldstein. Dopamine agonist potencies of ergolines. *Communs Psychopharmac.* **3:** 179–183, 1979.
7. Perry, K. W. and R. W. Fuller. Analysis of biogenic amine metabolites in rat brain by HPLC with electrochemical detection. *Soc. Neurosci. Abstr.* **5:** 349, 1979.
8. Solem, J. H. and T. Brinck-Johnsen. An evaluation of a method for determination of free corticosteroids in minute quantities of mouse plasma. *Scand. J. clin. Lab. Invest.* **17:** Suppl. 80, 1–14, 1965.