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# Stimulation of Prolactin Secretion by Metoclopramide in the Rat (39697)[1]

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Metoclopramide (2-methoxy-5-chloroprocainamide) is a procainamide derivative that has been used in Europe for 12 years as an anti-emetic and stimulant of smooth muscle of the upper gastrointestinal tract (1, 2). The drug, presently under investigation in the United States, is a potent stimulant of prolactin (PRL) secretion in man (3-5); PRL-stimulating doses (10-20 mg) of metoclopramide produce serum drug levels of about 100-200 ng/ml in humans (6). Although clinical evidence (4, 5) has suggested dopamine antagonism as a mechanism for metoclopramide-induced PRL secretion, there have been no direct tests of this hypothesis. In the present study, we describe experiments which lend support to a dopamine-antagonistic action of metoclopramide in promoting PRL secretion.

*Materials and methods. In vivo experiments.* Groups of six 200-g male Sprague-Dawley rats received intraperitoneal injections of 100 $\mu$g of metoclopramide dissolved in 0.5 ml of physiologic saline or saline alone. Sixty minutes after injection, the rats were killed by decapitation, and the trunk blood was collected for hormonal analysis.

*In vitro experiments.* Hormone secretion from isolated rat pituitary tissue was studied in a simple perifusion system previously described (7). In brief, two pituitary quarters, obtained from a 150-250-g male Sprague-Dawley rat, were placed on a wire grid in a stainless steel Millipore-Swinny filtration chamber and were immersed in a 37° water bath. Modified Gey and Gey buffer (7), equilibrated with 95% $O_2$-5% $CO_2$, was pumped through the chamber, and, after a 2.5-hr preincubation period, the effluent was collected in 2-ml (4 min) aliquots on a fraction collector for hormonal assay. Test substances dissolved in Gey and Gey buffer were introduced by means of a three-way stopcock. Mean effluent hormone levels during the final 20 min of the initial control period were compared with mean levels during the subsequent period of administration of test substances. At least three experiments using pituitaries from separate animals were performed for each concentration of test substance. Student's *t*-tests for paired or unpaired data were used, as appropriate, to assess the significance of observed changes in hormone levels.

*Hormone assays and reagents.* Rat serum and perifusion effluent PRL and growth hormone (GH) concentrations were measured using specific radioimmunoassays (7, 8). Reagents for both assays were supplied by Dr. A. F. Parlow, NIAMDD Hormone Distribution Program. The rat PRL standard used for radioimmunoassay (NIAMDD Rat PRL RP-1) had a bioassay potency of 11 IU/mg, whereas the GH standard (NIAMDD Rat GH RP-1) had a potency of 0.6 IU/mg. Crystalline metoclopramide was kindly supplied by the A. H. Robins Co., Richmond, Va., and dopamine hydrochloride was purchased from Sigma Chemical Company.

*Results. In vivo experiments.* Intraperitoneal administration of 100 $\mu$g of metoclopramide resulted in a significant ($P < 0.02$) increase in serum PRL levels 60 min later; the mean serum PRL was 85 ± 14 (SEM) ng/ml in the metoclopramide-treated rats compared to 31 ± 10 ng/ml in the saline-treated controls. Serum GH, in contrast, was not significantly affected; the mean serum GH following metoclopramide treatment, 39 ± 22 ng/ml, was similar to the value of 35 ± 16 ng/ml observed in saline-treated controls.

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*In vitro experiments.* Basal perifusion effluent hormone levels varied from experiment to experiment, depending on the size of the pituitary glands used. Perifusion experiments failed to demonstrate any direct effect of metoclopramide on PRL secretion over the concentration range of 0.2–200 μg/ml (Table I); Fig. 1 illustrates this lack of effect for perifusate metoclopramide concentrations of 20 μg/ml. Only a slight downward drift in perifusion effluent PRL levels, also seen during control periods, was observed during metoclopramide perifusion. In contrast, 5.5 m*M* theophylline regularly provoked PRL release (Fig. 1), demonstrating tissue responsiveness. GH responses to perifused metoclopramide were variable; while metoclopramide concentrations of 200 ng/ml produced no significant effect on GH levels, 20 μg/ml concentrations of the drug significantly ($P < 0.05$) lowered effluent GH concentrations.

Dopamine (1 μg/ml of dopamine hydrochloride = $5.3 \times 10^{-6}$ *M*) produced prompt and significant ($P < 0.01$) inhibition of PRL secretion from the perifused pituitary tissue (Fig. 2A and Table I); mean effluent PRL levels during dopamine administration were 40% of preceding control values. In the presence of metoclopramide (20 μg/ml), however, this inhibitory effect of dopamine was markedly blunted; although a small decrease in perifusate PRL levels occurred, this was indistinguishable from the slight fall observed with metoclopramide alone ($P > 0.2$; see Fig. 2B and Table I). Dopamine also produced a significant fall in perifusate GH levels; this effect was similarly blocked by metoclopramide (Table I).

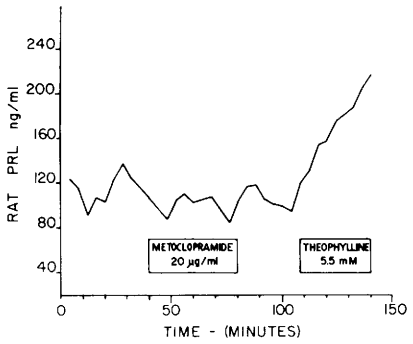


FIG. 1. Lack of effect of metoclopramide (20 μg/ml) on prolactin secretion from perifused rat hemipituitary. Effluent prolactin levels are plotted against time; a typical experiment is shown.

*Discussion.* The absence of direct stimulation of PRL secretion by metoclopramide in pituitary perifusion experiments *in vitro* coupled with the marked increment in serum PRL following *in vivo* administration suggests that the drug may promote PRL release through central nervous system (CNS)-dependent mechanisms. Such mechanisms may involve decreases in the hypothalamic release of prolactin-inhibiting factor, a substance whose secretion is thought to be enhanced by dopamine (9, 10). Alternatively, recent evidence suggests that dopamine itself may be secreted into pituitary portal blood (11) and may act directly on the pituitary to inhibit PRL secretion (12, 13). As a dopamine antagonist, metoclopramide could thus block these PRL-inhibitory effects of dopamine at the pituitary level, as we have demonstrated. Recent studies have shown that metoclopramide blocks the effect of dopamine on molluscan heart and intestine (14) and on the vascular

TABLE I. EFFECT OF METOCLOPRAMIDE ON BASAL AND DOPAMINE-INHIBITED PROLACTIN SECRETION FROM PERFUSED RAT PITUITARY

| | Perifusate hormone concentration (ng/ml; mean ± SEM) | | | |
|---|---|---|---|---|
| | Prolactin | | Growth hormone | |
| Drug | Control | Drug | Control | Drug |
| Metoclopramide (0.20 μg/ml) | 55.8 ± 15.9 (6)[a] | 48.0 ± 12.7 (6) | 619 ± 45 (3) | 465 ± 92 (3) |
| Metoclopramide (20 μg/ml) | 63.2 ± 14.9 (5) | 56.8 ± 11.3 (5) | 590 ± 138 (4) | 493 ± 79[b] (4) |
| Metoclopramide (200 μg/ml) | 37.6 ± 7.3 (3) | 32.3 ± 6.1 (3) | – | – |
| Dopamine-HCl (1 μg/ml) | 216 ± 19.2 (4) | 86.6 ± 9.8[c] (4) | 1702 ± 152 (4) | 871 ± 72[b] (4) |
| Dopamine-HCl (1 μg/ml) + Metoclopramide (20 μg/ml) | 142 ± 13.2 (4) | 120 ± 15.7[c] (4) | 1626 ± 332 (4) | 1545 ± 212 (4) |

[a] Numbers within parenthesis are number of experiments at each concentration.
[b] Significantly different from corresponding control period, $P < 0.05$.
[c] Significantly different from corresponding control period, $P < 0.01$.

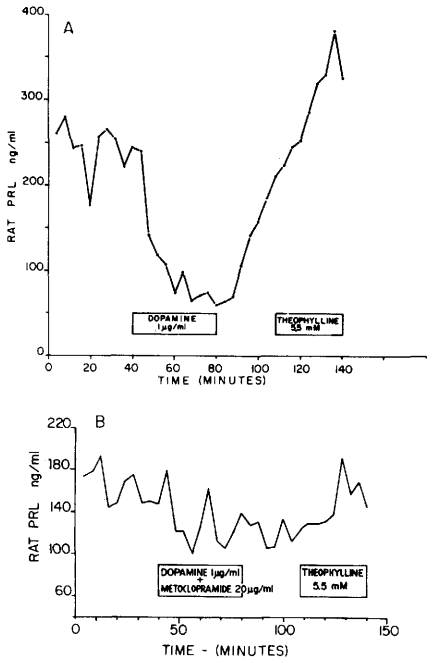


FIG. 2. (A) Inhibitory effect of dopamine (1 µg/ml) on prolactin release from perifused rat hemipituitary. (B) Blunting of prolactin inhibitory effect of dopamine (1 µg/ml) in the presence of metoclopramide (20 µg/ml). For both A and B, typical perifusion experiments are shown.

systems of the rat and cat (15), providing further support for a dopamine-antagonistic action of the drug. In either case, we have found no evidence for a direct stimulatory effect of metoclopramide on PRL release; available data therefore suggest that metoclopramide elevates serum PRL *in vivo* by reducing dopamine-mediated inhibition of prolactin secretion. Such a mechanism would also be consistent with observations of blunted PRL responses to metoclopramide in humans pretreated with L-DOPA (4, 5). Although our data support a pituitary site of dopamine antagonism, they do not rule out an additional or alternative hypothalamic site of action. The clinical observation of extrapyramidal side effects in patients receiving large doses of metoclopramide chronically (16, 17) supports a possible role for metoclopramide as a CNS antagonist of dopamine, since depletion of dopamine in the basal ganglia is thought to occur in Parkinson's disease (18).

Quijada *et al.* (19) have previously reported a modest inhibition by dopamine of rat GH secretion *in vitro*; our observations confirm these findings and further demonstrate that this effect, like the PRL-inhibiting actions of dopamine, is blocked by metoclopramide.

*Summary.* Metoclopramide, a procainamide derivative known to raise serum prolactin (PRL) levels in intact humans, produced a significant increase in serum PRL when administered intraperitoneally to male rats. In contrast, direct application of metoclopramide to the isolated rat pituitary in a perifusion system did not increase prolactin release *in vitro*. In this system, dopamine inhibited prolactin secretion. Perifusion with both metoclopramide and dopamine blocked the inhibitory effect of dopamine on prolactin release. This suggests that metoclopramide promotes PRL secretion by antagonism of dopamine-mediated CNS-dependent mechanisms. Although metoclopramide had no effect on serum growth hormone in the intact rat, the drug antagonized the growth hormone-inhibiting action of dopamine *in vitro*.

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