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Interaction of Morphine with the Cholinergic System on Prolactin Release (41105)

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Abstract. The effects of morphine, pilocarpine, and two anticholinergic drugs on serum prolactin (PRL) levels were studied in unanesthetized male rats, each previously implanted with an atrial cannula for collection of blood for radioimmunoassay. Morphine induced a threefold increase in serum PRL levels, whereas pilocarpine, a cholinergic agonist, significantly decreased serum PRL levels. When given together, the two drugs partially counteracted each other's effects on PRL release. Atropine, an anticholinergic drug, had no effect on serum PRL levels when injected alone, but when given together with morphine, significantly enhanced morphine induced PRL release. This enhancing effect of muscarinic blockade on morphine induced PRL release was confirmed in a second experiment by using scopolamine, another anticholinergic drug. These results suggest that the stimulating effect of morphine on PRL release may be mediated in part by a decreased activity in the central cholinergic system.

Endogenous opioid peptides and morphine (MOR) can induce release of prolactin (PRL), whereas naloxone, a specific opioid antagonist, can prevent stimulation of PRL release by the opiates (1–8). The mechanism(s) by which opiates increase PRL release have not been entirely elucidated, although it was reported that the opiates reduce dopamine and increase serotonin activity in the hypothalamus (9). Administration of cholinergic drugs, including pilocarpine (PILO) and physostigmine, were shown to decrease serum PRL levels (10). PILO also was reported to partially counteract MOR-induced stimulation of PRL release in rats (11). Since both the cholinergic system (10–12) and the endogenous opiates (1, 2, 4, 6, 7) appear to participate in regulating PRL release, it was of interest to compare the effects of administering MOR alone, together with the cholinergic agonist, PILO, or with the cholinergic antagonists, atropine and scopolamine, on PRL release in rats.

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Materials and Methods. *Animals.* Male Sprague–Dawley rats (Harlan Industries, Cumberland, Ind.), weighing 250–300 g, were housed in a temperature- (23 ± 1°) and light-controlled room (lights on from 0500 to 1900 hr). After arrival the animals were allowed several days to acclimatize, and fed food pellets (Ralston Purina Co., St. Louis, Mo.) and water *ad libitum*.

Drugs. Atropine sulfate (ATRO, Sigma Chemical Co., St. Louis, Mo.), morphine sulfate (MOR, Mallinkrodt Labs, St. Louis, Mo.), pilocarpine nitrate (PILO, Nutritional Biochemicals, Cleveland, Ohio), and scopolamine hydrobromide (SCOP, Sigma Chemical Co.) were dissolved in 0.87% NaCl solution (SAL) just before use.

Experiment I. Intracardiac venous cannulae were inserted under ether anesthesia 48 hr prior to experimentation. Each cannula, consisting of silastic tubing (Dow-Corning, Midland, Mich.), was inserted into the right external jugular vein 32 mm from the right atrium. The following treatments were administered to groups of 10 animals each: PILO, 10 mg/kg BW; ATROP, 10 mg/kg BW; MOR 10 mg/kg BW; MOR, 10 mg/kg + PILO, 10 mg/kg BW; MOR, 10 mg/kg + ATROP, 10 mg/kg BW. Control rats received an equivalent volume of saline (SAL) vehicle only.

All drugs and SAL alone were administered iv via cannulae immediately after the

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first blood sampling. Blood (0.5 ml) was collected in heparinized syringes 30, 60, and 120 min after drug administration. Samples were immediately centrifuged and the plasma was frozen for hormone assay. A volume of 0.5 ml of SAL was given via the cannula after each blood withdrawal.

Experiment II. Cannulated rats (six animals per group) were injected with MOR, MOR + ATROP, or MOR + SCOP. MOR and ATROP were administered in the same doses as in Experiment I and SCOP was given at a dose of 2 mg/kg BW. The experimental procedure was the same as in Experiment I, except that red cells were resuspended in SAL and returned via the cannula after withdrawal of each blood sample, as described previously (8).

Hormone assays and statistical procedure. Plasma PRL was determined by radioimmunoassay (RIA), using the double-antibody method of Niswender *et al.* (13) with an NIAMDD kit provided by Dr. A. F. Parlow (Torrance, Calif.). Results were expressed as nanograms of NIAMDD-RP-1 PRL per milliliter plasma. Analysis of variance and Student–Newman–Keul's test for multiple comparisons among groups were used to analyze the data. The level of significance chosen was $P < 0.05$.

Results. Results from Experiment I are summarized in Table I. SAL administration had no effect on serum PRL values. PILO significantly decreased serum PRL to values under 3 ng/ml by 30 min, and they remained lower than initial values through 120 min. The serum PRL levels of the PILO-treated rats also were significantly lower than in SAL-treated control rats 30, 60, and 120 min after drug administration. ATROP alone had no effect on serum PRL values, whereas MOR significantly increased serum PRL levels 30 and 60 min after injection. When MOR and PILO were administered together, PILO partially counteracted MOR-induced PRL release. On the other hand, ATROP significantly enhanced MOR-induced release of PRL throughout the 120 min after combined drug administration.

In Experiment II (Fig. 1), MOR alone raised serum PRL levels at 30 and 60 min after injection as in Experiment I. Both ATROP and SCOP significantly enhanced the rise in serum PRL produced by MOR throughout the 120-min period after drug injection.

Discussion. The results of this study show that MOR increased PRL release and PILO decreased PRL release in rats, in agreement with previous reports (1–8, 10, 11). The data also show that the effect of each drug on PRL release was partially counteracted when they were given in combination. Of greatest interest is the observation that ATROP and SCOP, both anticholinergic drugs, greatly enhanced the PRL stimulating action of MOR on PRL release. ATROP and SCOP each practically doubled the rise in serum PRL induced by MOR alone. This suggests that the stimulating action of MOR on PRL release may

TABLE I. EFFECTS OF MORPHINE AND CHOLINERGIC DRUGS ON SERUM PROLACTIN LEVELS

Experimental group[a]	Time (min)			
	0	30	60	120
SAL (controls)	19.5 ± 0.7[b]	20.7 ± 2.3	21.9 ± 3.2	17.3 ± 2.4
PILO, 10 mg/kg	16.1 ± 1.9	<3.0[c]	10.2 ± 0.7[c]	9.2 ± 1.0[c]
ATRO, 10 mg/kg	17.6 ± 1.3	21.4 ± 2.6	18.0 ± 2.3	15.3 ± 1.0
MOR, 10 mg/kg	18.9 ± 2.0	63.0 ± 5.0[c]	46.3 ± 6.9[c]	16.9 ± 0.5
MOR + PILO	18.9 ± 1.8	35.4 ± 6.5[c]	27.9 ± 4.0[c]	14.5 ± 2.1
MOR + ATRO	17.5 ± 1.8	165.4 ± 15.0[c]	91.3 ± 14.4[c]	35.4 ± 3.7[c]

Note. Intact male rats were used, and cannulae were implanted 2 days prior to experimentation. Drugs were injected via the cannula immediately after the first blood withdrawal, as described under Materials and Methods.

[a] n = 10 animals per group.

[b] Mean ± SE.

[c] Significantly different from initial (0) value and from corresponding control values, $P < 0.05$.

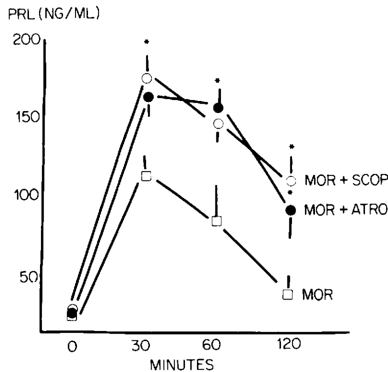

FIG. 1. Effect of Injection of MOR alone and together with ATRO or SCOP on serum PRL levels. n = 6 animals per group. Vertical lines = mean ± SE * $P < 0.05$ MOR + ATRO and MOR + SCOP vs MOR group.

be partially mediated by decreasing cholinergic activity in the hypothalamus (11). Earlier, our laboratory had shown that the inhibitory action of cholinergic drugs on PRL release in rats was mediated via the dopaminergic system, by increasing dopamine metabolism (9, 14). It is possible, therefore, that MOR acts first to reduce hypothalamic cholinergic metabolism, and this in turn, results in decreased dopamine metabolism, as previously observed after opiate administration (9, 14–16). Dopamine is considered by many investigators to be the primary inhibitor of PRL release, acting directly on the pituitary (17–20), but other agents also may be involved (10, 14, 19, 21). Cholinergic receptors have been demonstrated to be present in the anterior pituitary (22–25) and their role as nicotonic or muscarinic receptors was indicated by their interaction with various cholinergic agonists and antagonists (25). Direct inhibitory effects of cholinergic compounds on PRL release by anterior pituitary cell cultures also have been reported (22, 24).

Although ATROP alone did not alter basal PRL release at the dose given, in agreement with earlier reports (10, 14), the present observations raise the question as to whether the hypothalamic cholinergic system is activated by stimuli which normally stimulate PRL release. We previously observed that elevations of serum PRL levels induced by stress, suckling, and pseudopregnancy can be partially or completely counteracted by administering cholinergic drugs (Grandison and Meites, unpublished). Stimulation of PRL release by MOR was shown here to be similarly partially counteracted by the cholinergic agonist, PILO. This observation and the finding that ATROP and SCOP can greatly enhance MOR-induced PRL release, indicate that the cholinergic system is directly involved in PRL release. The present and related findings also suggest that acetylcholine may be an essential link in the inhibitory regulation of PRL release.

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