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Prolactin-Lowering Effect of Nomifensine in the Rat (40614)[1]

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It is controversial whether in the mammalian central nervous system (CNS) there exists a distinct peptidergic prolactin (PRL)-inhibiting factor (PIF) whose secretion is under dopaminergic control, or the PIF activity of the hypothalamus may be accounted for by brain dopamine (DA) (1, 2). Dopamine released from tuberoinfundibular dopaminergic nerve terminals into the hypophysial portal circulation (3) may act directly at the level of the anterior pituitary (AP), where receptor sites for DA have been located (4). Therefore, the study of the PRL-lowering effect of drugs capable of enhancing dopaminergic neurotransmission without directly affecting DA receptor sites located on the AP may be of value in elucidating the function of the "central" dopaminergic component involved in the control of PRL secretion. Nomifensine (Nom), a recently developed antidepressant drug (5), which has a pharmacologic profile somewhat different from that of the classic tricyclic antidepressants (6), appears to be a drug suitable for this purpose. Reportedly, it activates DA neurotransmission mainly by inhibiting DA reuptake (7, 8) and facilitating DA release (9), with no ability to affect directly DA receptor sites. This manuscript reports the results of experiments on the PRL-inhibiting effect of Nom in the rat.

Materials and methods. Animals. Adult male and female rats of the Sprague–Dawley (S.D.) strain (Nossan, Corezzana, Italy) weighing 250–300 g body wt were used in most of the experiments. All rats were housed in an air-conditioned room with controlled lighting (lights on 6 AM–8 PM) and fed a standard rat diet and water *ad libitum*. Rats were maintained in this environment for at least 3 days prior to experiments.

In the experiments in rats bearing an AP transplant under the kidney capsule (see below), hypophysectomized (hypox) female S.D. rats (110–130 g body wt) and intact female littermates, which served as pituitary donors, were used. Fifteen days following hypophysectomy by transauricular route (10), hypox rats received transplants equivalent to an entire AP under the kidney capsule. Following transplantation and before the experiments, animals were weighed daily; only animals which showed a constant body weight increase throughout this period were included in the study. Thirty female S.D. rats underwent bilateral ovariectomy and were used 15 days after surgery.

Experimental procedure. In vivo experiments. Experiments were performed starting at 8.30–9.30 AM or 3.00–4.00 PM (ovariectomized rats).

At different time intervals after drug treatments (see Results), rats were either killed by decapitation or sampled repeatedly by the retroorbital venous technique (11); blood was collected into heparinized tubes, plasma was then separated and stored at −20°, until assayed for PRL by radioimmunoassay (RIA) (see below). Ovariectomized (OVX) female rats were given a single injection of estradiol (E_2, 25 μg/kg body wt) and were sampled in the afternoon of the second and third postinjection days. In the experiments with the use of α-methyl-*p*-tyrosine, intact male rats were prepared with chronic jugular cannulae inserted into the right atrium and blood samples (0.3–0.4 ml) were taken from rats housed individually in small isolation cages using a procedure previously described (12).

In vitro experiments. Male rats of the S.D. strain (200–250 g body wt) were used as AP donors. Anterior pituitaries were hemisected and four hemipituitaries were placed in 2 ml of medium TC 199 (Difco Labs., Detroit, Mich). Incubation was carried out in a Dubnoff metabolic shaker under constant gassing

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with 95% O_2–5% CO_2 at 37°. After 30 min of preincubation, the medium was removed and pituitary tissue was placed in 2 ml of fresh medium containing either diluent or drugs under examination (see Results). After incubation for 4 hr, each AP fragment was blotted on filter paper and weighed. The medium was frozen and kept at −20° until assayed for PRL by RIA.

Drugs. The drugs employed in the present study were: nomifensine (8-amino-2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline) (Alival, Hoechst, Frankfurt, West Germany), reserpine (Serpasil, Ciba–Geigy, Origgio, Italy), α-methyl-*p*-tyrosine methylester (α-MpT, Prodotti Gianni, Milano, Italy), a blocker of catecholamine biosynthesis (13), dopamine (Farmacia Ospedale Maggiore, Milano, Italy), and 2-Br-α-ergocryptine (CB 154, Sandoz, Milano, Italy), which was used as a direct stimulant of DA receptors (14).

PRL radioimmunoassay. Prolactin was determined by a double-antibody radioimmunoassay, using the method of Niswender *et al.* (15). All results were expressed in ng/ml in terms of the NIH standard rat PRL-RP-1, whose potency is 11 IU/mg. The sensitivity of the PRL assay is 1.0 ng/ml; intrassay variability was 5% while repeated assay of a reference plasma showed an interassay variation of 6%. To avoid possible interassay variations all samples of each experiment were assayed in a single radioimmunoassay.

Plasma PRL levels were expressed as absolute values; in the *in vitro* experiments PRL concentration in the medium was expressed in ng/ml/mg fresh pituitary. In all experiments significance of differences between groups was calculated by Dunnett's *t* test preceded by ANOVA or paired Student's *t* test where applicable. A probability of $P < 0.05$ was chosen as statistically significant.

Results. PRL-lowering effect of Nom in intact male and in OVX-E_2 primed female rats. Nom administered at the dose of 10 mg/kg i.p. induced a significant decrease of baseline PRL levels in intact male rats; its effect was significantly different from that elicited by saline injection in control rats at the doses of 2.5–5.0 and 10 mg/kg i.p. (Table I).

In a second experiment the effect of Nom on the plasma PRL rise induced by E_2 in OVX rats was compared with that evoked in the same animals by CB 154. Nom at the dose of 5.0 and 10.0 mg/kg i.p. induced a significant decrease in baseline PRL levels 60 min after injection; its effect was significantly different from that elicited by saline injection in control rats at the dose of 10 mg/kg i.p. CB 154 decreased baseline PRL levels at the doses of 1.25, 2.5, and 5.0 mg/kg i.p. In spite of a consistent diminution of baseline plasma PRL in saline-treated rats, likely attributable to a spontaneous dampening of the afternoon peak of plasma PRL at 5–6 PM (16), the lowering effect of CB 154 was statistically significant at all three dose levels ($P < 0.05$ vs saline at 120 min) (Table II).

Effect of Nom on plasma PRL levels in reserpine-pretreated male rats. A single injection of reserpine (2.5 mg/kg i.p.) induced a significant rise of plasma PRL levels 18 hr later. Nom at the dose of 10 mg/kg significantly lowered baseline PRL values in diluent-pretreated animals and was able to counteract reserpine-induced hyperprolactinemia (Table III).

Effect of Nom on plasma PRL levels in reserpine-α-MpT-pretreated male rats. Administration of reserpine (2.5 mg/kg i.p.) plus

TABLE I. EFFECT OF NOMIFENSINE (Nom) ON PLASMA rPRL LEVELS IN MALE RATS

Treatment[a]	Doses	rPRL ng/ml[b] t_0	rPRL ng/ml[b] $t_{1\ hr}$	Δ From baseline levels
Saline	2 ml/kg ip	26.4 ± 4.4	25.3 ± 4.2	−1.2 ± 3.9
Nom	2.5 mg/kg ip	20.3 ± 3.8	12.5 ± 2.1[c]	−7.7 ± 4.4
Nom	5 mg/kg ip	17.2 ± 1.5	11.5 ± 1.8[c]	−5.7 ± 3.1
Nom	10 mg/kg ip	24.1 ± 4.4	8.8 ± 1.2[c, d]	−15.4 ± 4.7[c]

[a] Male S.D. rats (250–300 g) were sampled by the retroorbital venous technique at times 0 and 1 hr after Nom administration.

[b] Values represent the mean ± SEM of seven to eight determinations.

[c] $P < 0.05$ vs saline-treated animals.

[d] $P < 0.05$ vs baseline values.

TABLE II. EFFECT OF NOMIFENSINE (Nom) OR CB 154 ADMINISTRATION ON PLASMA rPRL LEVELS IN E_2-PRIMED OVARIECTOMIZED RATS

Groups[a]		rPRL ng/ml[b]		
		t_0	$t_{60\ min}$	$t_{120\ min}$
Saline	(2 ml/kg ip)	517 ± 146	397 ± 86	—
Nom	(2.5 mg/kg ip)	377 ± 57	416 ± 130	—
Nom	(5 mg/kg ip)	382 ± 55	165 ± 48[c]	—
Nom	(10 mg/kg ip)	567 ± 151	81 ± 15[c, d]	—
Saline	(2 ml/kg ip)	1149 ± 185	—	601.0 ± 105[c]
CB 154	(1.25 mg/kg ip)	476 ± 110[d]	—	38.8 ± 9.6[c, d]
CB 154	(2.5 mg/kg ip)	579 ± 112[d]	—	78.8 ± 37.1[c, d]
CB 154	(5 mg/kg ip)	521 ± 121[d]	—	59.3 ± 26.9[c, d]

[a] Ovariectomized S.D. rats (180–200 g) were given a single injection of E_2 (25 μg/kg s.c.) 15 days after surgery. Experiments were performed in the same animals starting at 3–4 PM of the second (Nom) and third (CB 154) day after E_2 injection. Rats were sampled by the retroorbital venous techniques immediately before and 1 (Nom) or 2 hr (CB 154) after drug administration.

[b] Values represent the mean ± SEM of six determinations.

[c] $P < 0.05$ vs baseline (paired t test).

[d] $P < 0.05$ vs respective saline.

TABLE III. EFFECT OF NOMIFENSINE (Nom) ON PLASMA rPRL LEVELS IN MALE RATS PRETREATED WITH RESERPINE

Pretreatment[a]		Treatment[a]		rPRL ng/ml[b]
Saline	(2 ml/kg i.p.)	Saline	(2 ml/kg i.p.)	23.1 ± 5.2
Reserpine	(2.5 mg/kg i.p.)	Saline	(2 ml/kg i.p.)	47.5 ± 7.4[c]
Saline	(2 ml/kg i.p.)	Nom	(10 mg/kg i.p.)	4.2 ± 1.8[c]
Reserpine	(2.5 mg/kg i.p.)	Nom	(10 mg/kg i.p.)	11.4 ± 1.9[d]

[a] Male S.D. rats (200–220 g) were pretreated 18 hr before Nom administration with reserpine or saline. Rats were sampled by the retroorbital venous technique 1 hr after Nom administration.

[b] Values represent the mean ± SEM of nine determinations.

[c] $P < 0.05$ vs saline-pretreated–saline-treated animals.

[d] $P < 0.05$ vs reserpine-pretreated–saline-treated animals.

α-MpT (200 mg/kg i.p.) resulted in a striking rise in baseline PRL levels which was present up to 90 min. The PRL-lowering effect of Nom, which was evident 60 and 90 min post-injection in the diluent-pretreated rats, was completely suppressed in reserpine-α-MpT-pretreated rats (Table IV).

Effect of Nom in hypox rats bearing an ectopic AP. From the results reported in Table V, it appears that administration of Nom at two dose levels, i.e., 5 and 10 mg/kg i.p., did not significantly diminish baseline PRL values 1 and 2 hr later. It must be noted, however, that in both experiments there was a trend toward lower PRL levels following administration of the higher dose of the compound.

Effect of Nom on pituitary PRL release in vitro. In a final experiment, the effect of two different doses of Nom was evaluated on the secretion of PRL from *in vitro* AP fragments and compared to that of equimolar doses of DA, a drug previously shown capable of inhibiting PRL release from *in vitro* AP (17). Nom was completely ineffective in inhibiting PRL release at both dose levels (5×10^{-7} and 5×10^{-6} M), whereas DA induced already its maximum inhibitory effect at the dose of 5×10^{-7} M (Table VI).

Discussion. Nomifensine effectively inhibited PRL release in animals with either basal or stimulated PRL secretion. This effect, likely resulting from nomifensine-induced activation of dopaminergic neurotransmission (7–9), is consistent with the reported PRL-lowering effect of dopaminergic agonists, such as bromocriptine (18), apomorphine (19), lisuride (16) piribedil (20), and the DA precursor, L-dopa (21). The PRL-lowering effect of the drug was rather slight in male rats in the unstimulated state since only the dose of 10 mg/kg was effective. In the same animal model bromocriptine strikingly lowers baseline PRL levels at the dose of 1 mg/kg (22). Instead, the PRL-suppressive effect of nomifensine was more clear cut

TABLE IV. EFFECT OF NOMIFENSINE (Nom) ON PLASMA rPRL LEVELS IN MALE RATS PRETREATED OR NOT WITH RESERPINE AND α-METHYL-P-TYROSINE (α-MpT)

Pretreatment[a]	Treatment[a]	rPRL ng/ml[b]					
		$t_{-45\ min}$	$t_{-30\ min}$	t_0	$t_{30\ min}$	$t_{60\ min}$	$t_{90\ min}$
Saline (2 ml/kg i.p.)	Saline (1 ml/kg i.v.)	26.3 ± 7.1	23 ± 6.0	19.4 ± 8.5	21.4 ± 8.4	14.6 ± 4.3	20.6 ± 5.0
Reserpine + α-MpT (2.5 mg/kg i.p.) (200 mg/kg i.p.)	Saline (1 ml/kg i.v.)	66.0 ± 15.0	81.7 ± 8.4	123.7 ± 18.5[d, e]	176.0 ± 28.0[d, e]	159.0 ± 18.4[d, e]	120.0 ± 19.4[d]
Saline (2 ml/kg i.p.)	Nom (10 mg/kg i.v.)	40.3 ± 15.0	22.8 ± 3.6	18.4 ± 8.8	7.8 ± 2.6	5.6 ± 1.8[c]	2.9 ± 1.8[c]
Reserpine + α-MpT (2.5 mg/kg i.p.) (200 mg/kg i.p.)	Nom (10 mg/kg i.v.)	69.0 ± 12.7	95.5 ± 23.0	137.0 ± 32.0[d, e]	114.0 ± 29.4[e]	116.0 ± 25.0[d, e]	118.0 ± 25.0[d]

[a] Male S.D. rats (250–280 g) permanently cannulated in the jugular vein were sampled at different times before and after Nom or saline administration. Nom was administered at t_0, reserpine 18 hr, and α-MpT 30 min before Nom injection.

[b] Values represent the mean ± SEM of six to eight determinations.

[c] $P < 0.05$ vs baseline values (paired t test).

[d] $P < 0.05$ vs saline-pretreated–saline-treated animals at the corresponding time.

[e] $P < 0.05$ vs saline-pretreated–Nom-treated animals at the corresponding time.

TABLE V. EFFECT OF NOMIFENSINE (Nom) ON PLASMA rPRL LEVELS IN HYPOPHYSECTOMIZED RATS BEARING AN ECTOPIC PITUITARY

Groups[a]	rPRL ng/ml[b]		
	t_0	$t_{1\ hr}$	$t_{2\ hr}$
Expt I			
Saline (2 ml/kg i.p.)	59.0 ± 17.8	54.5 ± 15.3	58.2 ± 21.9
Nom (5 mg/kg i.p.)	79.5 ± 30.6	63.2 ± 18.3	49.5 ± 21.1
Nom (10 mg/kg i.p.)	122.5 ± 47.6	48.2 ± 13.5	35.5 ± 6.2
Expt II			
Saline (2 ml/kg i.p.)	43.0 ± 12.7	44.5 ± 14.5	45.6 ± 14.5
Nom (5 mg/kg i.p.)	47.0 ± 17.2	24.1 ± 6.4	23.1 ± 7.3
Nom (10 mg/kg i.p.)	66.3 ± 39.2	18.8 ± 2.6	19.3 ± 3.8

[a] Female S.D. rats (110–130 g body wt) received an entire AP fragment under the kidney capsule 15 days before.

[b] Values represent the mean ± SEM of six to eight determinations. Rats were sampled by the retroorbital venous technique immediately before and 1 and 2 hr after Nom administration.

when baseline PRL levels had been raised artificially, i.e., in the OVX-rats given estrogens. In view of the reported mechanism of action of the drug (7–9), it may be proposed that in OVX-E_2-primed rats, despite the presence of hyperprolactinemia, activation of DA neurotransmission is nevertheless competent to suppress PRL secretion.

In keeping with the report of Horowski and Gräf (23), who demonstrated that the PRL-inhibiting effect of another indirect DA agonist drug, i.e., amphetamine, is maintained in reserpine-pretreated rats, nomifensine lowered plasma PRL when given in animals pretreated with reserpine. This finding is at variance with behavioral studies showing that the same dose of reserpine, i.e., 2.5 mg/kg, completely antagonized the stereotyped licking/biting activity of nomifensine (9), although that due to amphetamine was maintained (24).

TABLE VI. EFFECT OF NOMIFENSINE (Nom) OR DOPAMINE (DA) ON rPRL RELEASE FROM *IN VITRO* PITUITARIES

Groups[a]	Doses (M)	rPRL ng/ml/mg pituitary
Control	—	203.1 ± 28.3
Nom[b]	5×10^{-7}	158.4 ± 25.5
Nom	5×10^{-6}	207.0 ± 8.1
DA	5×10^{-7}	93.2 ± 16.2[c]
DA	5×10^{-6}	84.0 ± 12.7[c]

[a] Each group represents the mean of five samples. For each sample four hemipituitaries incubated for 4 hr in 2 ml of TC 199 with or without drugs were used.

[b] As diluent for Nom a solution of HCl 2 N diluted 1:10.000 with TC 199 was used.

[c] $P < 0.05$ vs control.

In our study, inhibition of catecholamine synthesis in reserpine-treated animals by α-MpT abolished the PRL-lowering effect of nomifensine. In view of the different mecha-

nisms whereby reserpine and α-MpT affect brain DA stores (25, 26), these data would indicate that nomifensine causes the release of DA from a newly synthesized pool of the neurotransmitter. The same conclusion has been recently reached by Gudelski and Porter (27) for amphetamine, in experiments involving direct measurement of DA in pituitary stalk plasma.

A presynaptic action of nomifensine is also implied by its failure to inhibit PRL release from AP fragments *in vitro* and to lower the high baseline PRL levels of hypox rats bearing an ectopic pituitary. It is a common notion in fact that in mammals the AP does not contain the presynaptic neuronal component (28), through which activation of the postsynaptic catecholamine receptor occurs physiologically. However, analysis of the data reported in Table V reveals a trend toward lower PRL levels in the hypox-transplanted rats given the highest drug dose. This might imply a direct, though minor, action of the drug or a drug metabolite (29) on pituitary postsynaptic DA receptors, which in a gland disconnected from the CNS are exquisitely sensitive to dopaminergic stimulation (30, 31). Alternatively, nomifensine by acting on peripheral sites might have induced an increase in DA arterial blood levels.

The central stimulant effects of nomifensine in rodents have been causally related to the very potent inhibitory effect of the drug on the uptake mechanism in DA synaptosomes (7, 8). The present study points to a more significant role for the DA-releasing action of nomifensine. In fact, if one admits that the drug action with respect to PRL secretion is exerted at the level of the median eminence (ME), it appears unlikely that nomifensine may act to inhibit the uptake of DA which is released into the portal circulation. It is conceivable in fact the presynaptic capture of the released amine may not play at ME level a major role in the removal of the neurotransmitter, a task which is better accomplished by the downward flow of the portal blood. However, the possibility cannot be ignored that nomifensine may facilitate DA neurotransmission by blocking DA uptake at a central synapse between a dopaminergic neuron and another neuron (PIF?) of unknown nature on which postsynaptic DA receptors are located. Direct measurements of DA in portal blood before and after nomifensine treatment are needed to resolve this problem.

The availability of drugs such as nomifensine, whose action rests on the integrity of dopaminergic transmission in the CNS, offers a valid pharmacologic means to probe hyperprolactinemic states due to an altered CNS control. In this vein, results obtained in hyperprolactinemic subjects have shown that nomifensine is capable of lowering plasma PRL in "functional" hyperprolactinemia but fails to do so in patients bearing PRL-secreting adenomas (23). The dopaminergic defect present in the CNS of the tumor subjects (33, 34) is likely the cause of such behavior.

Summary. The effect of nomifensine (Nom), a new antidepressant agent which facilitates dopaminergic neurotransmission by blocking the reuptake of dopamine (DA) into nerve terminals and by promoting DA release, was studied on plasma prolactin (PRL) levels in the rat. Nom reduced baseline plasma PRL levels in untreated or reserpine-pretreated male rats and in ovariectomized, estrogen-primed female rats. Nom did not alter plasma PRL levels in reserpine-α-methyl-*p*-tyrosine-pretreated male rats and in hypophysectomized female rats bearing a pituitary transplant under the kidney capsule nor inhibited PRL release from *in vitro*-incubated pituitaries. The availability of drugs such as Nom, whose action rests on the integrity of dopaminergic transmission in the CNS, without involving direct stimulation of pituitary DA receptors, offers a valid means to investigate CNS function in hyperprolactinemic states.

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