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Effects of minaprine, a novel antidepressant, on prolactin secretion in the rat

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Abstract—The effect of minaprine, a novel psychotropic drug with antidepressant properties, on prolactin secretion has been investigated in the rat. On intraperitoneal administration (10 and 20 mg kg^{-1}) it significantly decreased basal prolactin levels. In contrast, both haloperidol (1 mg kg^{-1} i.p.) and morphine (20 mg kg^{-1} i.p.) increased serum prolactin levels and daily treatment with oestradiol (100 μg kg^{-1} s.c.) for 4 days also elevated the levels. Minaprine at a dose of 20 mg kg^{-1} failed to antagonize the elevation of serum prolactin levels induced by these drugs. The results imply that minaprine may not exert a direct inhibitory action on prolactin secretion at the pituitary gland.

Minaprine, 4-methyl-6-phenylpyridazin-3-yl(2-morpholinoethyl)amine, is chemically unrelated to other known psychotropic drugs (Wermuth & Exinger 1972), and has recently been shown to have a therapeutic effect in human depressions (Passeri et al 1985). Minaprine was reported to antagonize prochloperazine-induced catalepsy and potentiate amphetamine-induced stereotyped behaviour, suggesting that the drug may enhance dopaminergic transmission (Bizière et al 1982, 1984). Minaprine also produced an increase of acetylcholine content and a decrease of choline content and inhibited acetylcholinesterase activity in the striatum. In addition, the increase of striatal acetylcholine levels produced by minaprine was partially prevented by pimozide, a dopamine receptor antagonist. From these results, Garattini et al (1984) proposed that minaprine acts partially as a dopaminergic agonist in increasing acetylcholine content in the striatum. Furthermore, Ferretti et al (1984) described that minaprine has no direct action on dopaminergic receptors, but it affects dopamine metabolism by acting, at least partially, at presynaptic sites through the inhibition of monoamine oxidase (MAO) activity. Thus, it is conceivable that minaprine modifies dopaminergic neuron activity.

Secretion of prolactin in the rat is regulated mainly by neurotransmitters and hypothalamic hormones which have both inhibitory and stimulatory effects. The tuberoinfundibular dopamine system, especially, has been proposed to participate in the regulation of prolactin secretion (MacLeod 1976; Moore & Demarest 1982). Actually, serum prolactin levels are decreased by bromocriptine, a dopamine receptor agonist, and increased by haloperidol, a dopamine receptor antagonist (Moore & Demarest 1982; Yamada et al 1986). Moreover, prolactin secretion is enhanced by various drugs such as morphine (Koenig et al 1979), thyrotropin-releasing hormone (TRH) (Bowers et al 1971; Yamada et al 1986) and oestrogen (Ratner et al 1963; Gudelsky et al 1981; Yamada et al 1985).

The present study was therefore undertaken to investigate the effects of minaprine on basal prolactin levels and the increases of prolactin levels induced by some prolactin releasing agents.

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Methods

Male Wistar rats (220–250 g) supplied from Kyudo Animal Laboratory (Kumamoto, Japan) were maintained in an air-conditioned laboratory at a temperature of 21 ± 1 °C in a 12 h light–dark cycle (light on 0700h). Commercial food (CE-2, Clea Ltd, Japan) and tap water were freely available except during the period of experiments.

Blood was taken from the trunks of decapitated rats and centrifuged at 3000*g* for 30 min. Serum prolactin levels were measured by radioimmunoassay based on protocols and reagents kindly supplied from the National Hormone and Pituitary Agency (rat prolactin RP-3 standard and anti-rat prolactin serum-9) (Yamada et al 1986).

The following drugs were used: minaprine dihydrochloride (kindly supplied by Sanofi, Montpellier, France), haloperidol (Serenace Injection, Dainippon, Osaka, Japan), morphine hydrochloride (Dainippon, Osaka, Japan) and β-oestradiol 3-benzoate (Sigma, St Louis, Missouri, USA). Oestradiol dissolved in sesame oil was administered daily by subcutaneous injection for 4 days, and the last treatment being 24 h before death. The other drugs dissolved or diluted in saline were injected intraperitoneally (i.p.) 30 min before killing. Animals receiving injection of saline served as controls. Doses are expressed in terms of the salts where available, or in terms of the base.

Prolactin levels in serum were expressed as the mean value. Statistical analysis was done using a one-way analysis of variance followed by the Dunnett's *t*-test (difference between a control and all means) or Tukey's test (difference between all means) (Winer 1971).

Results

Prolactin level in serum observed after intraperitoneal injection of saline was 7·1 ± 0·7 ng mL^{-1}. A low dose of minaprine (5 mg kg^{-1} i.p.) did not change basal prolactin levels, but high doses (10–20 mg kg^{-1} i.p.) significantly decreased the levels (Table 1).

Table 1. Effects of minaprine on basal prolactin levels in male rats.

Drugs (mg kg^{-1})	Prolactin (ng mL^{-1})
Saline	7·1 ± 0·7
Minaprine 5	7·1 ± 0·8
Minaprine 10	3·3 ± 0·3**
Minaprine 20	4·6 ± 0·4*

Each value represents the mean ± s.e. of serum prolactin levels from 10 rats.
*$P < 0.05$, **$P < 0.01$; significant difference from saline-injected control group.

As shown in Table 2, treatment with haloperidol (1 mg kg^{-1} i.p.), morphine (20 mg kg^{-1} i.p.) or oestradiol (100 μg kg^{-1} × 4 days, s.c.) increased prolactin levels. However, the increases in prolactin levels induced by these drugs were not affected by

minaprine, at a dose of 20 mg kg^{-1}, which decreased the basal prolactin levels. In the additional experiments, minaprine (20 mg kg^{-1} i.p.) also failed to affect the slight, but significant, increase in prolactin levels induced by haloperidol at the lower doses of 0·1 and 0·2 mg kg^{-1} (i.p.), prolactin levels after haloperidol alone at each dose and in combination with minaprine being 24·5 ± 2·4 (n = 9), 36·7 ± 5·4 (n = 8), 29·8 ± 2·6 (n = 9) and 37·7 ± 4·5 ng mL^{-1} (n = 9), respectively.

Table 2. Effects of minaprine on the increases of prolactin secretion induced by haloperidol, morphine or oestrogen in male rats.

Drugs	Prolactin (ng mL^{-1})
Saline + saline	9·6 ± 0·8
Minaprine + saline	4·0 ± 0·2*
Saline + haloperidol	43·6 ± 2·2*
Minaprine + haloperidol	52·5 ± 4·5*
Saline + morphine	53·5 ± 6·5*
Minaprine + morphine	47·5 ± 7·5*
Oestrogen + saline	35·5 ± 3·4*
Oestrogen + minaprine	31·8 ± 2·5*

Rats were decapitated 30 min after injection of saline (1 mL kg^{-1} i.p.), minaprine (20 mg kg^{-1} i.p.), haloperidol (1 mg kg^{-1} i.p.) or morphine (20 mg kg^{-1} i.p.) and 24 h after the last treatment with oestradiol (100 μg kg^{-1} × 4 days, s.c.).

Each value represents the mean ± s.e. of serum prolactin levels from 10 rats.

* $P < 0·01$; significant difference from saline plus saline-injected group.

Discussion

The results indicate that minaprine reduced basal prolactin levels, but was unable to decrease the elevation of prolactin levels induced by haloperidol, morphine or oestrogen. It is well known that prolactin secretion is tonically inhibited by dopamine released from tuberoinfundibular dopaminergic neurons and is thereby stimulated by dopamine receptor antagonists, such as haloperidol, which block dopamine receptors at the anterior pituitary (MacLeod 1976; Moore & Demarest 1982; Sarkar et al 1984; Yamada et al 1986). On the other hand, it has been reported that minaprine may enhance dopaminergic transmission since it antagonized prochloperazine-induced catalepsy and potentiated amphetamine-induced stereotyped behaviour (Bizière et al 1982, 1984). However, minaprine affected neither the release of dopamine nor [^{3}H]spiperone binding to striatal membrane (Bizière et al 1983; Ferretti et al 1984). Recently, minaprine has been found to inhibit type A MAO mainly after being converted into active metabolites (Kan et al 1986). From these results, it has been proposed that minaprine appears to stimulate the dopaminergic system by increasing availability of dopamine via the inhibition of MAO activity (Ferretti et al 1984; Kan et al 1986). Therefore, it is assumed that the decrease of prolactin secretion induced by minaprine may be at least in part due to the increasing availability of dopamine at the pituitary.

Morphine has been reported to stimulate prolactin secretion by an activation of 5-HT neuron activity as well as the inhibition of tuberoinfundibular dopaminergic neuron activity but not by acting directly on the pituitary (Muraki & Tokunaga 1978; Koenig et al 1979). Oestrogen also stimulates the production of prolactin from the anterior pituitary mammotrophs (Ratner et al 1963; Yamada et al 1985) and enhances the prolactin-releasing effects of TRH, which are due to increased production of inositol triphosphate via a stimulation of TRH receptors at the pituitary (Martin 1983; Rebecchi et al 1983). Indeed, in the present experiment, prolactin secretion was markedly increased by treatment with haloperidol, morphine or oestrogen. The increases of prolactin release induced by these drugs have been reported to be inhibited by dopamine receptor agonists such as apomorphine and bromocriptine (Muraki & Tokunaga 1978; Moore & Demarest 1982) or muscarinic receptor agonists such as pilocarpine (Muraki et al 1979; Hylka et al 1986; Kumagai et al 1987). On the other hand, minaprine was also reported to increase the 5-HT levels and decrease 5-hydroxyindoleacetic acid levels in the striatum, though it did not affect the release of 5-HT (Bizière et al 1983). Moreover, minaprine inhibited acetylcholinesterase activity in the striatum (Garattini et al 1984). Thus, in addition to modification of dopamine neuron activity, minaprine is proposed to modify 5-HT- and cholinergic neuron activities (Bizière et al 1983; Garattini et al 1984). However, in our study, the increases of prolactin secretion induced by haloperidol, morphine and oestrogen were not affected by minaprine. To the best of our knowledge, the interference in minaprine metabolism by these agents has not been reported. Accordingly, minaprine may not have a direct inhibitory action on prolactin release at the anterior pituitary mammotrophs.

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Effects of AN-132 and quinidine, antiarrhythmic agents, on plasma digoxin concentrations in rats

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Abstract—The effects of AN-132, 3-(diisopropylaminoethylamino)-2′,6′-dimethylpropionanilide·$2H_3PO_4$, on chloroform-induced arrhythmias and plasma digoxin concentrations have been compared with those of quinidine in rats. AN-132 (0·01–3 mg kg^{-1}) administered orally significantly inhibited the incidence of cardiac arrhythmias in a dose-related fashion. A single dose of digoxin (1 mg kg^{-1}) given orally for 7 consecutive days was followed, on day 8, orally by digoxin alone, or together with AN-132 (50, 100 and 200 mg kg^{-1}) or quinidine (25 and 50 mg kg^{-1}). The AUC_{0-24} and C_{max} of plasma digoxin were enhanced significantly by co-administration of quinidine, but not by AN-132.

Although use of antiarrhythmic drugs such as quinidine during maintenance digoxin therapy has now become common in patients with cardiac disease, severe adverse effects have often been reported (Bigger & Hoffman 1985). Quinidine and some other antiarrhythmics are known to alter the pharmacokinetics of digoxin, and increase plasma/serum digoxin levels, heightening the risk of adverse reactions (Ejvinson 1978; Leahey et al 1978; Chen & Friedman 1980; Weeks et al 1986). AN-132 is a novel diamine derivative which is under development as an orally efficacious antiarrhythmic drug, and its potent antiarrhythmic activity has been evidenced in several animal models (Sakai et al 1987).

The aim of the present study was to examine the antiarrhythmic effect of AN-132, and compared with quinidine, its effect on digoxin pharmacokinetics in rats. A further aim was to predict whether a single dose of AN-132 affects the plasma digoxin level when co-administered with oral digoxin.

Methods

Production of chloroform arrhythmias. Male Sprague-Dawley rats, 100 g, were deprived of food overnight before the experiment but had free access to water. According to a small modification of the method of Erker & Baker (1980), the animals were given intramuscularly 20 mg kg^{-1} aminophylline, and 30 min later placed for 50 s in a 4 L covered beaker containing 200 mL of chloroform. Although most of the chloroform was absorbed by gauze pads on the bottom of the beaker, excess liquid chloroform was always present to maintain a fairly constant vapour pressure. At respiratory arrest, the rats were removed from the beaker, their abdomens and thoraxes were opened without touching the hearts, and the cardiac ventricles were visually examined. The heart was classified as being in a state of spontaneous ventricular arrhythmia if the arrhythmia lasted at least 5 s after the ventricles were exposed for visual examination. If the ventricles were contracting in a coordinated manner they were stimulated by quick pinches with a metal forceps. Drug solutions were given orally in a volume of 2 mL kg^{-1} for 20 s, 30 min before exposure to chloroform.

Pharmacokinetics. Male Sprague-Dawley rats, 340 to 380 g, received digoxin (1 mg kg^{-1}) orally, once a day (between 0900 and 1000h) for 7 days. Just before dosing, blood samples (0·15 mL) were withdrawn from the tail vein, using heparinized syringes, for digoxin determination, and centrifuged at 3000 rev min^{-1} for 10 min with a Hitachi Refrigerated Centrifuge (05 PR-22). Plasma was separated, transferred to test tubes and frozen at −20 °C until assayed (within 2 days). The animals were fasted overnight from the evening on day 7, but had free access to tap water. On day 8, the animals were divided into 6 groups (n = 7). Group I was treated with digoxin (1 mg kg^{-1}) alone, Group II with digoxin and quinidine (25 mg kg^{-1}), Group III with digoxin and quinidine (50 mg kg^{-1}), Group IV with digoxin and AN-132 (50 mg kg^{-1}), Group V with digoxin and AN-132 (100 mg kg^{-1}), and Group VI with digoxin and AN-132 (200 mg kg^{-1}). Venous blood samples (0·15 mL) were withdrawn before drug treatment. Thereafter, blood samples (0·15 mL) were withdrawn from the tail vein into heparinized syringes 1, 2, 4, 6 and 24 h after oral dosing, and stored as previously. The drugs were suspended in 3% gum arabic solution, and given by gavage in a volume of 1·0 mL. Plasma digoxin concentrations were measured in duplicate using a radioimmunoassay kit (digoxin ^{125}I kit, Dainabot, Japan). Radioactivity was determined by means of an Aloka γ-counter (model 600). Plasma samples were diluted 10 times with 0·9% NaCl (saline), then 0·1 mL of each sample was used for assay. The area under the plasma concentration time curve

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