Neuroleptic-induced prolactin rise: Influence of pharmacological alterations of different neurotransmitter systems

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Summary. Oral administration of 2 neuroleptic drugs, haloperidol and LR511 induced in male rats a marked, dose-dependent and sustained rise of plasma prolactin.

There is now general agreement that prolactin (PRL) secretion is mainly under inhibitory hypothalamic control[1,2], although the chemical nature of the prolactin release inhibiting factor (PIF) is still unknown. Dopamine (DA) is the key transmitter amine in this inhibitory process and the possibility exists that DA itself is the inhibiting factor acting on the pituitary cells[3,4]. Blockade of DA receptors by neuroleptic drugs induces a dose-dependent increase in PRL release[5].

In addition to DA, other neurotransmitters appear to be involved in the control of PRL release. Evidence has been given for a central serotoninergic (5-HT) mechanism playing a stimulatory role in the release of the hormone[6,7], while for the cholinergic (ACh) system controversial data have been reported[8]. The same is true for gamma-aminobutyric acid (GABA), for which both an inhibitory role, directly exerted at the level of the pituitary lactotrophs[9,10], and a stimulatory action[11,12] have been proposed. Histamine (H) has been suggested to stimulate PRL release through H_1 receptors[13] and to inhibit PRL release through H_2 receptors[14].

The purpose of this work was to examine the influence of drugs affecting various neurotransmitter systems on the hyperprolactinemia induced by neuroleptic drugs. Haloperidol (HAL) and LR511, a novel psychotropic drug recently described[15], were administered in combination with drugs acting at central cholinergic, serotoninergic, histaminergic and GABAergic systems.

Materials and methods. Male Sprague-Dawley rats (Lusofarmaco, Milan, Italy) weighing 170–180 g were housed in a temperature (23 ± 1 °C) and humidity (60 ± 5%) controlled room with lights on from 08.00 to 18.00 h. Altromin R chow and water were supplied ad libitum. Animals were killed by decapitation; the blood was collected into heparinized tubes, immediately centrifuged and the plasma separated and kept frozen at −20 °C until assayed. PRL was measured by radioimmunoassay (RIA) using the method of Niswender et al.[16]. All results were expressed in ng/ml in terms of the NIH standard rat prolactin RP-1, the potency of which is 11 IU/mg. The sensitivity of the assay was 1.0 ng/ml. Intraassay 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 RIA.

Single treatment. Haloperidol (HAL, Jannsen, Beerse, Belgium) and 4-p-fluorophenyl-5-N (N'-methoxyphenyl) piperazino-ethyl-4-oxozolin-2-one (LR511, Lusofarmaco, Milan, Italy) were suspended at different concentrations in 10% arabic gum and administered orally by gavage at the constant volume of 5 mg/kg. Dose-response and time-course studies were carried out on 8–16 rats for each group.

Combined treatment. Atropine sulphate (ATR, Merck, Darmstadt, FRG, 5 mg/kg), diphenhydramine hydrochloride (DPH, Prodotti Gianni, Milan, Italy, 10 mg/kg) and diazepam (DIA, Prodotti Gianni, Milan, Italy, 10 mg/kg) were solu-dispersed in arabic gum and administered i.p.; muscimol hydrobromide, (MUS, synthetized by P. Krogsgaard-Larsen, 2 mg/kg) and apomorphine hydrochloride (APO, Sandoz, Milan, Italy, 3 mg/kg) were dissolved in distilled water and injected i.v.; metergoline (MCE, Farmitalia, Milan, Italy, 2.5 mg/kg) was dissolved in distilled water containing maleic acid and ethanol and administered p.o. Drug doses are expressed as free base. All these substances were administered alone or simultaneously with the neuroleptics 1 h prior to sacrifice. A few animals were given 2 injections of APO at an interval of 40 min. For each group 12–16 rats were used. A 2-way analysis of variance and a Dunnett's t-test were used to compare plasma PRL levels between controls and the different treatment groups; data were subjected to $\log_{10}$ transformation before analysis.

Results and discussion. Single treatment. 1 h after the administration of increasing doses of LR511 and HAL there was a dose-related increase in plasma PRL although with LR511 the dose-response curve was flat (table). A time-course study revealed that the maximum plasma PRL level occurred around the 1st h of treatment with both neuroleptics (figure 1). Prolactin levels were already considerably lower at the 2nd h, but still above control baseline levels after 5 h. No significant effect on plasma PRL was observed 1 h after administration of ATR, DPH, DIA and MCE. On the contrary, MUS and APO, reduced baseline PRL levels (table).

Combined treatment. ATR and DPH did not modify the rise in plasma PRL induced by either neuroleptic, whereas APO prevented this increase (figure 2, upper panel). MUS

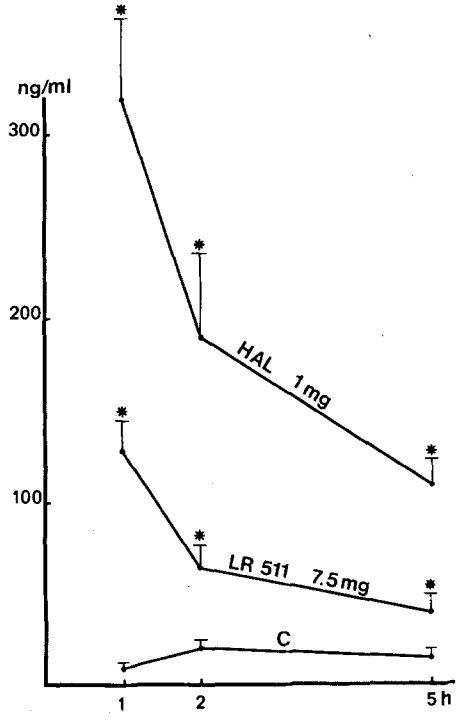

Fig. 1. Plasma PRL levels (Mean ± SEM) in male rats 1, 2, 5 h after the oral administration of HAL or LR511. C = Control; HAL = haloperidol; * = $p < 0.01$ vs controls.

and DIA significantly reduced HAL-induced but not LR511-induced plasma PRL rise; MCE, instead potentiated the hyperprolactinemic effect of LR511 and failed to modify that of HAL (figure 2, lower panel).

The peak PRL response present 1 h after oral administration of HAL (1 mg/kg) and the persistence of the effect during the 5 h of the experiments are in good agreement with data previously reported in male rats[17]. LR511 also induced a rise in plasma PRL, but the effect evoked by this drug was weaker than that of HAL and a poor dose-response correlation was observed. The lower hyperprolactinemic potency of LR511 than of HAL is consistent with the lower activity of the former in affecting other behavioral and biochemical indices reflecting anti-dopaminergic activity[15,18].

Of the many drugs used in this study only APO, a highly selective DA receptor agonist[19], and MUS, a specific agonist of GABAergic receptors[20], decreased baseline PRL levels significantly. Interestingly, while evidence has been given for the existence at pituitary level of receptors for both DA[21] and GABA[22], receptors for 5-HT, H and ACh are apparently lacking[2]. It would thus appear that only drugs capable of stimulating directly pituitary receptors are able to influence baseline hormone secretion.

Many reports in the literature[23-25] indicate that the major control of PRL secretion by DA is exerted at the level of 'peripheral' pituitary sites, with no or minor contributions by DA receptors located in the central nervous system (CNS). Consequently, HAL and LR511 could increase PRL release by blocking tonic inhibition by DA of pituitary lactotrophs. Consistent with this view is the finding that drugs such as MCE, DPH and ATR for which only a CNS site of action can be envisaged[2] failed to antagonize the PRL-releasing effects of LR511 and/or HAL and that APO and MUS were effective in this sense. The partial block of HAL-induced hyperprolactinemia and the reduction of basal prolactinemia (although not statistically significant in the present experiments) produced by DIA should most probably be related to the enhancing effect of benzodiazepines on GABAergic function, which has been demonstrated at various sites in the CNS[26]. 2 possible mechanisms of action may be envisaged. DIA may affect the pituitary lactotrophs indirectly through the tubero-infundibular DA (TIDA) system. In fact, in contrast to their effect on the nigro-striatal and meso-limbic DA systems, benzodiazepines increase the turnover of DA in the TIDA system[27], probably because of different synaptic connections between GABA neurons and DA neurons in these systems. The increased activity of TIDA neurons after DIA would result in an increased release of DA, which could partially overcome the neuroleptic-induced block of DA receptors in the pituitary. It has been shown recently that GABA originating from GABA-ergic nerve terminals in the median eminence, is released into the hypophyseal portal blood and transported to the pituitary where, by interacting with specific GABA receptors[22], it inhibits PRL release. Thus, as an alternative hypothesis, an interaction between DIA and GABA at the pituitary level may be postulated. Diazepam competitively interacting with the binding of GABA modulin, an endogenous inhibitor of high affinity binding of GABA[29], may render pituitary GABA receptors more sensitive to endogenous GABA transported via the hypophyseal portal blood. The effect of muscimol on PRL secretion would be more marked than that of DIA because muscimol is a very potent stimulant of GABA receptors and, in contrast to diazepam, does not require the presence of endogenous GABA to exert its effect. Indirect support for the involvement of GABA in the effect of DIA is the finding that DIA and MUS behaved similarly on PRL release; both were capable of blocking HAL-induced hyperprolactinemia and were unable to counteract the PRL-release induced by LR511. The latter finding has no valid explanation; the hypothesis of a stronger binding to pituitary DA receptors by LR511 with respect to HAL, in view of its lower potency as PRL releaser, appears unlikely. The potentiation by MCE of the PRL-releasing effect of LR511 and its failure to affect HAL-induced hyperprolac-

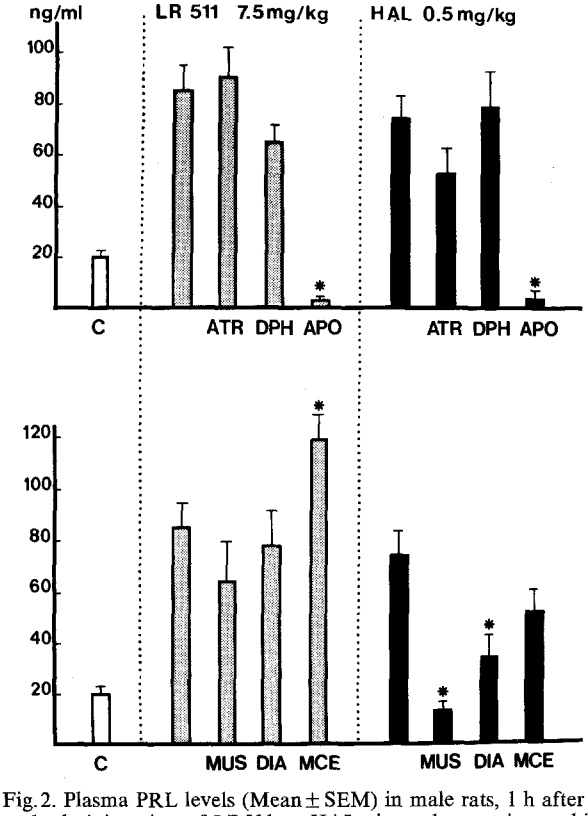

Fig. 2. Plasma PRL levels (Mean ± SEM) in male rats, 1 h after the oral administration of LR511 or HAL given alone or in combination with drugs affecting different central neurotransmitter systems. C = Control; HAL = haloperidol; ATR = atropine; DPH = diphenhydramine; APO = apomorphine; MUS = muscimol; DIA = diazepam; MCE = metergoline; * = $p < 0.01$ vs neuroleptic alone treated rats.

Plasma PRL levels in male rats 1 h after the administration of haloperidol (HAL) or LR511 or other drugs affecting CNS neurotransmission

Drugs	Dose (mg/kg)	Route of administration	PRL (ng/ml)[a]
Vehicles	–	p.o./i.p.	24.0± 2.8[b]
Haloperidol	0.5	p.o.	143.6±32.2*
	1.0	p.o.	318.6±45.6*
LR511	1.9	p.o.	110.9± 6.5*
	3.8	p.o.	147.7± 6.7*
	7.5	p.o.	159.1±12.7*
	15.0	p.o.	174.8± 6.8*
Atropine	5.0	i.p.	19.5± 6.6
Diphenhydramine	10.0	i.p.	29.4± 6.2
Diazepam	10.0	i.p.	12.8± 3.8
Muscimol	2.0	i.v.	7.2± 0.8*
Metergoline	2.5	p.o.	14.6± 5.6
Apomorphine	3.0	i.v.	3.5± 1.6*
	3.0±3.0[c]	i.v.	3.5± 1.2*

[a] Mean ± SEM of 8–16 determinations. [b] Pooled data from control rats treated with different vehicles (28 determinations). [c] Rats received a 2nd APO injected 40 min later. * $p < 0.01$ vs vehicle.

tinemia again shows a difference in the mode of action of the 2 neuroleptics, without offering a meaningful interpretation. Enhancement by MCE of the PRL-releasing effect of another antidopaminergic drug i.e., sulpiride also has been reported in rats[30]. In behavioral studies, MCE, and also ATR, were able to potentiate the effect of LR511 but not that of HAL on the inhibition of conditioned avoidance response[31], results which are in part reminiscent of those obtained in our study.

In contrast to the observed failure of MCE to affect the PRL-releasing effect of HAL is the reported ability of alleged 5-HT antagonists, i.e., SQ 10631 and methysergide, to inhibit the elevation in PRL levels induced by another anti-dopaminergic drug, pimozide[32]. It has to be noted, however, that the neuronal specificy of methysergide as a selective 5-HT antagonist has been questioned[2], and a direct stimulation of pituitary DA receptors by this drug has been demonstrated[33,34].

In conclusion our results demonstrate that: a) HAL-induced PRL release is antagonized by DA agonists and, in addition by drugs capable of enhancing (pituitary?) GABAergic function, thus suggesting an important role for GABA in the control of PRL secretion; b) the novel neuroleptic drug, LR511, behaves differently from HAL as far as PRL secretion is concerned, so that its PRL-releasing effect cannot be accounted for by a simple blockade of DA receptors.

1 J. Meites, in: Nonstriatal Dopaminergic Neurons, Adv. Biochem. Psychopharmac., vol. 16, p. 139. Ed. E. Costa and G.L. Gessa, Raven Press, New York 1977.
2 E.E. Müller, G. Nisticò and U. Scapagnini, Neurotransmitters and Anterior Pituitary Function. Academic Press, New York 1978.
3 R.M. Mac Leod and J.E. Lehemeyer, Endocrinology *94,* 1077 (1974).
4 C.J. Shaar and J.A. Clemens, Endocrinology *95,* 123 (1974).
5 H.Y. Meltzer and V.S. Fang, Arach. gen. Psychiatr. *33,* 279 (1976).
6 H.K. Lu and J. Meites, Endocrinology *93,* 152 (1973).
7 I. Gil-Ad, F. Zambotti, M.O. Carruba, L. Vicentini and E.E. Müller, Proc. Soc. exp. Biol. Med. *151,* 512 (1976).
8 L. Grandison and J. Meites, Fed. Proc. *35,* 306 (1976).
9 A.V. Schally, T.W. Redding, A. Arimura, A. Dupont and G.L. Linthicum, Endocrinology *100,* 681 (1977).
10 E.E. Müller, D. Cocchi, V. Locatelli, P. Krogsgaard-Larsen, F. Bruno and G. Racagni, in: GABA-Neurotransmitters, p. 318. Ed. P. Krogsgaard-Larsen, J. Scheel-Krüger and H. Kofold. Munksgaard, Copenhagen 1979.
11 T. Mioduszewski, L. Grandison and J. Meites, Proc. Soc. exp. Biol. Med. *151,* 44 (1977).
12 J.G. Ondo and K.A. Pass, Endocrinology *99,* 1248 (1976).
13 C. Rivier and W. Vale, Endocrinology *101,* 508 (1977).
14 M.C. Arekelian and C. Libertun, Endocrinology *100,* 890 (1977).
15 G.B. Fregnan and M. Vidali, Pharmacology *15,* 485 (1977).
16 G.D. Niswender, C.L. Chen, A.R. Midgley, Jr, J. Meites and S. Ellis, Proc. Soc. exp. Biol. Med. *130,* 793 (1969).
17 R. Ohman, M. Larsson, I.M. Nilsson, J. Engel and A. Carlsson, Naunyn-Schmiedebergs Arch. Pharmac. *299,* 105 (1977).
18 G.B. Fregnan and R. Porta, Arzneimittel-Forsch., in press (1980).
19 N.-E. Andén, A. Rubenson, K. Fuxe and T. Hökfelt, J. Pharm. Pharmac. *18,* 630 (1968).
20 D.R. Curtis, A.W. Duggan, D. Felix and G.A.R. Johnston, Brain Res. *32,* 69 (1971).
21 H. Kimura and R.M. Mac Leod, 57th A. Meet. endocr. Soc. New York, p. 94 (1975).
22 L. Grandison and A. Guidotti, Endocrinology *105,* 754 (1979).
23 N. Ben-Jonathan, C. Oliver, H.J. Weiner, R.S. Mical and J.C. Porter, Endocrinology *100,* 452 (1977).
24 C.Y. Cheung and R.L. Weiner, Endocrinology *99,* 914 (1976).
25 R.M. Mac Leod and I.S. Login, in: Nonstriatal Dopaminergic Neurons, Psychopharmac., vol. 16, Adv. Biochem. p. 147. Ed. E. Costa and G.L. Gessa, Raven Press, New York 1977.
26 W. Haefely, P. Pok, R. Schaffner, H.H. Keller, L. Pieri and H. Möhler, in: GABA-Neurotransmitters, p. 357. Ed. P. Krogsgaard-Larsen, J. Scheel-Krüger and B. Kofold. Munksgaard, Copenhagen 1979.
27 P. Lidbrink, H. Corrodi, K. Fuxe and L. Olson, in: The Benzodiazepines, p. 203. Ed. S. Garattini, E. Mussini and L.O. Randall. Raven Press, New York 1973.
28 G. Racagni, J.A. Apud, V. Locatelli, D. Cocchi, G. Nisticò, R.M. Di Giorgio and E.E. Müller, Nature, Lond. *281,* 575 (1979).
29 A. Guidotti, G. Toffano, M. Baraldi, J.P. Schwartz and E. Costa, in: GABA-Neurotransmitters, p. 406. Ed. P. Krogsgaard-Larsen, J. Scheel-Krüger and B. Kofold. Munksgaard, Copenhagen 1979.
30 M. Hoffmann, F. Battaini, P.F. Spano and M. Trabucchi, 6th Int. Congr. Pharmac., Paris 1978, Abstr., p. 929.
31 T. Chieli and G.B. Fregnan, Pharmac. Res. Commun. *11,* 617 (1979).
32 D.M. Lawson and R.R. Gala, Endocrinology *98,* 42 (1976).
33 S.W.J. Lamberts and R.M. Mac Leod, Endocrinology *100,* 287 (1978).
34 L. Krulich, R.J. Coppings, S.M. Mc Cann and M.A. Mayfield, Life Sci. *23,* 1665 (1978).

Immunization of female rabbits against testosterone stimulates testosterone accumulation by isolated ovarian follicles[1]

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Summary. Ovarian follicles isolated from female rabbits after active immunization against testosterone-3-oxime bovine serum albumin produced more testosterone than similar follicles from controls.

Immunization of animals in order to study the role of hormones is widely accepted as a useful tool in reproductive endocrinology. Although testosterone is predominantly a male sex hormone it may have an important role in ovarian physiology[3-7]. In a previous study[6] it was found that immunization of female rabbits against testosterone-3-oxime bovine serum albumin (T-3-BSA) resulted in increased levels of testosterone in circulation, but this could have been due to increased binding to antibodies, as noted, or to decreased metabolic clearance rate as observed in male rabbits[8]. In view of the marked hypertrophy of the interstitial cells observed in T-3-BSA immunized rabbits[7] our working hypothesis was that this cell layer could be another source of testosterone.