Br. J. clin. Pharmac. (1983), **16**, 471–475

PROLACTIN SECRETION IN MAN: A USEFUL TOOL TO EVALUATE THE ACTIVITY OF DRUGS ON CENTRAL 5-HYDROXYTRYPTAMINERGIC NEURONES. STUDIES WITH FENFLURAMINE

A. QUATTRONE, G. TEDESCHI, U. AGUGLIA, F. SCOPACASA[1], G.F. DI RENZO[2] & L. ANNUNZIATO[2]

Department of Neurology, Faculty of Medicine, University of Naples, [1]Department of Endocrinology, Faculty of Medicine and [2]Department of Pharmacology, Faculty of Medicine, University of Naples, Naples, Italy

1 Acute oral administration of various doses of fenfluramine, a 5-HT releaser, induced a dose-related increase of PRL secretion in nine healthy volunteers.

2 Fenfluramine reached the maximum effect on PRL secretion at 4 h after its administration. This effect was already significant at 2 h and lasted till 8 h.

3 Metergoline, a 5-HT receptor blocker, when administered alone, decreased serum PRL levels in six healthy subjects. The pretreatment with this drug significantly antagonized the PRL-releasing action of fenfluramine (60 mg) suggesting that the effect of fenfluramine on PRL release may be mediated through a 5-HT mechanism in the brain.

4 These findings suggest the possibility that serum PRL levels in humans may represent a useful tool to evaluate, *in vivo*, the activity of drugs possessing putative 5-hydroxytryptaminergic properties.

Keywords prolactin 5-HT fenfluramine

Introduction

Several reports suggest that brain 5-hydroxytryptamine (5-HT) may play a stimulatory role in regulating prolactin (PRL) secretion in experimental animals. It has been reported that 5-HT precursors, such as L-tryptophan and 5-hydroxytryptophan (5-HTP), significantly increased plasma PRL levels in rats (Lu & Meites, 1973; Marchlewska-Koj & Krulich, 1975) while substances which deplete brain 5-HT content, such as *p*-chlorophenylalanine (PCPA) and 5,7-dihydroxytryptamine (5,7-DHT), have been found to decrease PRL secretion in the same animal species (Quattrone *et al.*, 1978; Wuttke *et al.*, 1977). Recently, it has also been shown that quipazine and (+)-fenfluramine, two 5-HT agonists (Fuxe *et al.*, 1975b; Garattini *et al.*, 1975; Samanin *et al.*, 1976), significantly stimulated PRL release in rats by acting through a 5-HT mechanism in the brain (Quattrone *et al.*, 1978, 1979a). Thus the evidence supports the stimulatory role of central 5-HT neurones in the control of PRL release. As for the mechanism by which brain 5-HT stimulates PRL secretion, studies have suggested the hypothesis that central 5-hydroxytryptaminergic neurones may act indirectly, through an inhibition of hypothalamic tuberoinfundibular dopaminergic neurones (TIDA) (Quattrone *et al.*, 1979b).

The role of brain 5-HT in regulating PRL release in man is not yet clear. Contradictory results have been reported with drugs believed to exert a stimulatory action on 5-HT neurones. Kato *et al.* (1974) found that oral 5-HTP administration was able to significantly increase PRL release in healthy subjects and Woolf & Lee (1977) observed a significant elevation of plasma PRL levels when the administration of tryptophan was followed by a water load. On the other hand, Handwerger *et al.* (1975), Beck-Peccoz *et al.* (1976), Wiebe *et al.* (1977), by giving 5-HT precursors, failed to show modifications of PRL release. Similar results have been obtained in man by Parati *et al.* (1980) with quipazine, a 5-HT agonist.

The present experiments were undertaken in order to further clarify the possible role of central 5-HT neurones in the control of PRL secretion. We investigated: (i) the effect of fenfluramine, a potent 5-HT releaser (Fuxe *et al.*, 1975b; Garattini *et al.*, 1975), on PRL secretion in healthy volunteers; (ii) the possibility that changes in serum PRL levels induced by this drug were mediated through a 5-HT mechanism in the brain.

Methods

All subjects were drug-free healthy volunteers. Informed consent was obtained from each of them. All the experiments were performed in the morning after an overnight fast.

Blood samples were taken from the antecubital vein by venepuncture. Serum was immediately separated and stored at −20°C until assayed.

Dose-response experiment

Nine subjects (six males and three females weighing 60–80 kg), aged 25–30 years, took part in the study. The women were in their early follicular phase of normal ovarian cycles.

Fenfluramine (Ponderal, Servier) tablets were given orally at 09.00 h.

Blood samples were always taken at 09.00 h (time 0) and 13.00 h (4 h). Treatments consisted of five single oral doses of fenfluramine (20–40–60–80–100 mg) which were administered on five different days. Each experimental day was followed by a 7 days washing-out period. Baseline values were obtained, at 09.00 h and 13.00 h, the day before the first drug administration.

Time-course experiment

Six males (weighing 65–80 kg) aged 27–40 years, took part in the study. Baseline values were obtained on day 1; on day 2, subjects received at 09.00 h, after the last baseline sample was taken, a single oral dose of fenfluramine (100 mg). Baseline samples were taken on day 1 at 09.00 h, 10.00 h, 11.00 h, 13.00 h, 17.00 h and at 09.00 h of the day 2; blood samples after drug administration were taken at 09.00 h, 10.00 h, 11.00 h, 13.00 h, 17.00 h and at 09.00 h of the day 3.

Fenfluramine-metergoline interaction

Six males (weighing 65–80 kg), aged 27–40 years, took part in the study. Treatments were given orally according to the following procedure: day 1, drug-free; day 2, fenfluramine (60 mg) at 09.00 h; day 3, metergoline (2 mg, Liserdol, Farmitalia) at 08.30 h; day 4, metergoline (2 mg) at 08.30 h and then fenfluramine (60 mg) at 09.00 h. Each experimental day was followed by a 3 days washing-out period.

Blood samples were always taken at 09.00 h and 13.00 h.

Analytical procedure

Serum PRL was evaluated by radioimmunoassay (RIA) (Sinha *et al.*, 1973) using the reagents supplied by Biodata. The minimum detectable serum PRL was 1.5 ng/ml and the mean coefficient of interassay variation was 10%.

All samples were analyzed in the same RIA in order to avoid the interassay variation.

Statistical analysis

The data of the dose-response experiment and the data of the time-course experiment were analyzed by ANOVA randomized block design and Duncan's new multiple range test.

The data of the metergoline-fenfluramine experiment were analyzed by two-way ANOVA and by Tukey's HSD test.

Results

Dose-response experiment (Figure 1)

No significant difference was found between 09.00 h and 13.00 h in baseline serum PRL levels (7.2 ± 1.1 and 7.5 ± 0.9 ng/ml respectively). Oral administration of fenfluramine, 20 and 40 mg, did not produce

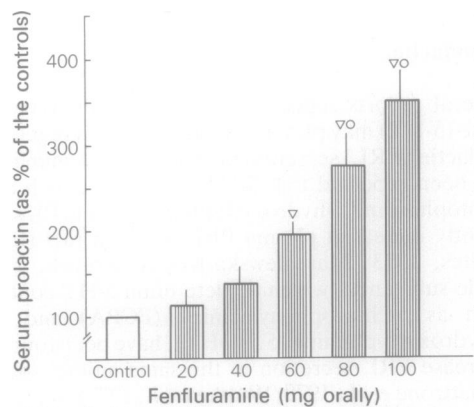

Figure 1 Effect of various doses of fenfluramine on serum PRL levels expressed as a percentage of the controls. Each column represents the mean±s.e. mean of nine subjects.
$\Delta P < 0.01$ when compared with controls (dose 0)
O $P < 0.01$ when compared with 60 mg dose
(ANOVA randomized block design and Duncan's new multiple range test)

any significant effect on serum PRL levels whereas a dose-related increase of PRL secretion occurred with 60, 80 and 100 mg doses.

In these experiments no significant difference was found, at any time, between males and females (data not shown).

Time-course experiment (Figure 2)

Fenfluramine reached the maximum effect on PRL secretion 4 h after its administration. This effect was already significant at 2 h and lasted till 8 h.

Fenfluramine-metergoline interaction (Table 1)

Oral administration of metergoline (2 mg) produced a significant decrease of serum PRL levels. The pretreatment with this drug significantly antagonized the PRL-releasing action of fenfluramine.

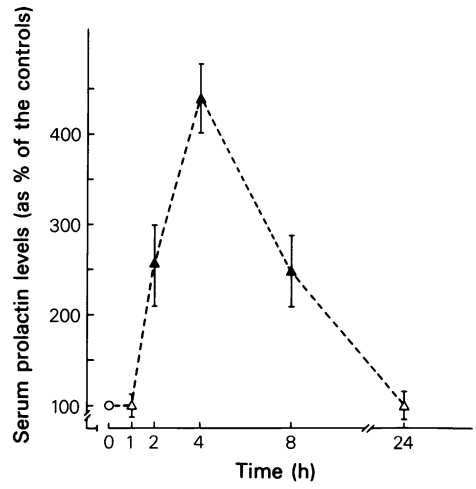

Figure 2 Time-course of fenfluramine (100 mg orally) on serum PRL levels. Solid symbols represent those values which are statistically significantly different ($P < 0.01$) from control levels (ANOVA randomized block design and Duncan's new multiple range test). Baseline values were obtained on day 1 at 09.00 h, 10.00 h, 11.00 h, 13.00 h, 17.00 h and at 09.00 h of day 2. Fenfluramine was administered in the same subjects, at 09.00 h of day 2, immediately after the last baseline sample was taken; blood samples were taken at 1, 2, 4, 8 and 24 h after drug administration.

Discussion

The present data show that fenfluramine induces dose-related increases in serum PRL levels in healthy volunteers. Fenfluramine is an anorectic drug which is known to activate 5-HT transmission in the brain either by stimulating the release of 5-HT or by inhibiting the reuptake of this amine at the presynaptic level (Fuxe *et al.*, 1975b; Garattini *et al.*, 1975). This drug has been shown to markedly stimulate PRL secretion in experimental animals by acting through a 5-HT mechanism in the brain. Quattrone *et al.* (1978, 1979a) have found that the PRL-releasing action of fenfluramine was abolished in rats pretreated either with substances aimed at decreasing brain 5-HT content, such as PCPA and 5,7-DHT, or with metergoline, a 5-HT receptor blocker (Mawson & Whittington, 1970; Fuxe *et al.*, 1975a).

In the present experiments, fenfluramine at doses of 60, 80 and 100 mg, significantly increased serum PRL levels in healthy subjects whereas it was ineffective at 20 and 40 mg doses. The peak serum PRL levels were observed 4 h after oral administration of fenfluramine. This is in agreement with other studies (Garattini & Caccia, 1979) showing that peak plasma fenfluramine levels were observed 4 h after oral administration of the drug in healthy volunteers.

Metergoline, when administered alone, significantly

Table 1 Effect of metergoline on the increase in serum PRL levels (mean ± s.e. mean) induced by fenfluramine.

Treatment	*Dose (mg orally)*	*Serum PRL levels (ng/ml)* 09.00 h	13.00 h
Placebo	—	6.9 ± 0.8	7.1 ± 0.9
Fenfluramine	60	8.0 ± 1.1	15.8 ± 1.2*
Metergoline	2	6.8 ± 1.0	4.0 ± 0.5*
Metergoline + fenfluramine	2 + 60	7.1 ± 1.1	3.9 ± 0.4**

Each value is the mean ± s.e. mean from six subjects.
*=$P < 0.01$ when compared with the placebo (Tukey's HSD test)
**=F for the interaction between metergoline and fenfluramine was highly significant ($P < 0.01$ two-way ANOVA)

decreased serum PRL levels in men. It is well known that this drug is able to inhibit PRL secretion after acute or short-term administration in normal subjects (Ferrari *et al.*, 1976), in puerperal women (Crosignani *et al.*, 1978) and in most hyperprolactinaemic patients (Ferrari *et al.*, 1978), this effect being usually attributed to the block of 5-HT receptors in the brain induced by this drug. In the present study, the pretreatment with metergoline markedly antagonized the PRL-releasing action of fenfluramine, suggesting that the effect of this latter drug on PRL release may be mediated via a 5-HT mechanism in the brain.

Although further information is needed on the functional link between 5-HT and PRL in the brain, the present findings indicate that central 5-hydroxytryptaminergic neurones may stimulate PRL secretion in man.

It is well known that the TIDA pathway tonically inhibits PRL release both in animals and in humans (MacLeod, 1976; Langer *et al.*, 1977), and that this effect is most probably exerted at the pituitary level (Shaar & Clemens, 1974; MacLeod, 1976). Thus, dopamine agonists markedly inhibit PRL release (Smalstig *et al.*, 1974) while dopamine synthesis inhibitors or blockers of dopamine receptors all stimulate PRL secretion (MacLeod, 1976; Meltzer *et al.*, 1978). On the basis of these results, the PRL response test is commonly used in evaluating relative clinical potency of drugs which interfere with dopaminergic neurones. As for 5-HT regulation of PRL release, our data suggest the possibility that serum PRL levels in humans may represent a useful tool to evaluate, *in vivo*, the activity of drugs possessing putative 5-hydroxytryptaminergic properties. Such an approach is valuable because nearly all the techniques currently used to measure the effect of drugs on central 5-hydroxytryptaminergic neurones are not applicable to humans.

References

BECK-PECCOZ, P., FERRARI, C., RONDENA, M., PARACCHI, A. & FAGLIA, G. (1976). Failure of oral 5-Hydroxytryptophan administration to affect prolactin secretion in man. *Hormone Res.*, **7**, 303–307.

CROSIGNANI, P.G., LOMBROSO, G.C., CACCAMO, A., RESCHINI, E. & PERACCHI, M. (1978). Suppression of puerperal lactation by metergoline. *Obstet. Gynecol.*, **51**, 113–115.

FERRARI, C., PARACCHI, A., BECK-PECCOZ, P. & FAGLIA, G. (1976). Effect of two serotonin antagonists on prolactin and thyrotrophin secretion in man. *Clin. Endocrinol.*, **5**, 575–578.

FERRARI, C., CALDARA, R., RAMPINI, P., TELLOLI, P., ROMUSSI, M., BERTAZZONI, A., POLLONI, G., MATTEI, A. & CROSIGNANI, P.G. (1978). Inhibition of prolactin release by serotonin antagonists in hyperprolactinemic subjects. *Metabolism*, **27**, 1499–1504.

FUXE, K., AGNATI, L. & EVERITT, B. (1975a). Effects of methergoline on central monoamine neurones. Evidence for a selective blockade of central 5-HT receptors. *Neurosci. Letters*, **1**, 283–286.

FUXE, K., FARNEBO, L.O., HAMBERGER, B. & OGREN, S.O. (1975b). On the *in vivo* and *in vitro* actions of fenfluramine and its derivatives on central monoamine neurons, especially 5-hydroxytryptamine neurons, and their relation to the anorectic activity of fenfluramine. *Postgrad. med. J.*, **51**, suppl. 1., 35–45.

GARATTINI, S., BUCZKO, W., JORI, A. & SAMANIN, R. (1975). On the mechanism of action of fenfluramine. *Postgrad med. J.*, **51**, Suppl. 1, 27–35.

GARATTINI, S. & CACCIA, S. (1979). Comparison of the plasma levels of fenfluramine in rats after a toxic dose and in man after a maximal therapeutic dose. *Toxicol. Letters*, **3**, 285–290.

HANDWERGER, S., PLONK, J.W., LEBOVITS, H.E., BIVENS, C.H. & FELDMAN, J.M. (1975). Failure of 5-hydroxytryptophan to stimulate prolactin and growth hormone secretion in man. *Hormone Metab. Res.*, **7**, 214–216.

KATO, Y., NAKAI, Y., IMURA, H., CHIHARA, K. & OHGO, S. (1974). Effect of 5-hydroxytryptophan (5-HTP) on plasma prolactin levels in man. *J. clin. Endocr. Metab.*, **38**, 695–697.

LANGER, G., SACHAR, E.J. & GRUEN, P.H. (1977). Human prolactin responses to neuroleptic drugs correlated with antischizophrenic potency. *Nature*, **266**, 639–640.

LU, H.K. & MEITES, J. (1973). Effects of serotonin precursors and melatonin on serum prolactin release in rats. *Endocrinology*, **93**, 152–155.

MACLEOD, R.M. (1976). Regulation of prolactin secretion. In *Frontiers in Neuroendocrinology*, vol. 4, eds. Martini, L. & Ganong, W.F., pp. 169–194. New York: Raven Press.

MARCHLEWSKA-KOJ, A. & KRULICH, L. (1975). The role of central monoamines in the stress-induced prolactin release in the rat. *Fed. Proc.*, **34**, 252.

MAWSON, C. & WHITTINGTON, H. (1970). Evaluation of the peripheral and central antagonistic activities against 5-hydroxytryptamine of some new agents. *Br. J. Pharmac.*, **39**, 223P.

MELTZER, H.Y., GOODE, D.J. & FANG, V.S. (1978). The effect of psychotropic drugs on endocrine function. I. Neuroleptics, precursors and agonists. In *Psychopharmacology: A Generation of Progress*, eds. Lipton, M.A., DiMascio, A. & Killam, K.F., pp. 509–529. New York: Raven Press.

PARATI, E.A., ZANARDI, P., COCCHI, D., CARACENI, T. & MULLER, E.E. (1980). Neuroendocrine effects of quipazine in man in health state or with neurological disorders. *J. Neural. Transm.*, **47**, 273–297.

QUATTRONE, A., DI RENZO, G.F., SCHETTINI, G., TEDESCHI, G. & SCOPACASA, F. (1978). Increased plasma prolactin levels induced in rats by d-fenfluramine: relation to central serotonergic stimulation. *Eur. J. Pharmac.*, **49**, 163–167.

QUATTRONE, A., SCHETTINI, G., DI RENZO, G.F., TEDESCHI, G. & PREZIOSI, P. (1979a). Effect of midbrain raphe lesion or 5,7-dihydroxytryptamine treatment on the prolactin-releasing action of quipazine and d-fenfluramine in rats. *Brain Res.*, **174**, 71–79.

QUATTRONE, A., SCHETTINI, G., DI RENZO, G.F. &

PREZIOSI, P. (1979b). Pharmacological evidence of an interaction between serotonergic and dopaminergic neurons in the control of prolactin secretion in male rats. *Arch. int. Pharmacodyn. Ther.*, **238**, No. 1, 42–49.

SAMANIN, R., BERNASCONI, S. & QUATTRONE, A. (1976). Antinociceptive action of quipazine: relation to central serotonergic receptor stimulation. *Psychopharmacologia (Berl.)*, **46**, 219–222.

SHAAR, C.J. & CLEMENS, J.A. (1974). The role of catecholamines in the release of anterior pituitary prolactin *in vitro*. *Endocrinology*, **95**, 1202–1212.

SINHA, A.N., SELBY, F.W., LEWIS, U.J. & VANDERLAAN, W.G. (1973). A homologous radioimmunoassay for human prolactin. *J. clin. Endocrinol. Metab.*, **36**, 509–516.

SMALSTIG, E.B., SAWYER, B.D. & CLEMENS, J.A. (1974). Inhibition of rat prolactin release by apomorphine *in vivo* and *in vitro*. *Endocrinology*, **95**, 123–129.

WIEBE, R.H., HANDWERGER, S. & HAMMOND, C.B. (1977). Failure of L-tryptophan to stimulate prolactin secretion in man. *J. clin. Endocrinol. Metab.*, **45**, 1310–1312.

WOOLF, P.D. & LEE, L. (1977). Effect of the serotonin precursor, tryptophan, on pituitary hormone secretion. *J. clin. Endocrinol. Metab.*, **45**, 123–133.

WUTTKE, W., BJORKLUND, A., BAUMGARTEN, H.G., LACHENMAYER, L., FENSKE, M. & KLEMM, H.P. (1977). De- and regeneration of brain serotonin neurons following 5,7-dihydroxytryptamine treatment: effects on serum LH, FSH and prolactin levels in male rats. *Brain Res.*, **134**, 317–331.

(Received March 31, 1983, accepted July 4, 1983)