EFFECTS OF TWO SUBSTITUTED BENZAMIDES, TIAPRIDE AND SULTOPRIDE, ON GONADOTROPHINS AND PROLACTIN

By

M. L’Hermite, R. M. MacLeod and C. Robyn

ABSTRACT

The acute effects in the human of tiapride and sultopride, two new substituted benzamides related to sulpiride, were studied with respect to gonadotrophins (LH and FSH) and prolactin (PRL) secretion. Two different groups of 3 normal men and 3 normally cycling women received im injections of either 100 mg sultopride or 200 mg tiapride. Five min after the injection of either psychotropic drug, the serum PRL concentration increased significantly (P < 0.001 by variance analysis) and reached maximal values by 30 min and remained elevated for at least 6 h; women tended to release more prolactin than men.

Although tiapride (500 nm) had no direct effect on the in vitro synthesis or secretion of prolactin, the drug blocked the inhibitory action of dopamine (500 nm) on the secretion of prolactin. Rats bearing a transplantable prolactin-secreting pituitary tumour MtTW15 have extremely high serum prolactin and through an autofeed mechanism the host’s pituitary gland is suppressed. The in vitro synthesis and secretion of prolactin by these rats’ pituitary gland is decreased. The in vivo administration of tiapride to these tumour-bearing rats restored the in vitro secretion of prolactin to control values. The in vitro data support the concept that tiapride increases prolactin secretion directly at the pituitary level by blocking the inhibitory activity of dopamine; an additional effect at a higher level can not, however, be ruled out.

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In vitro in the human, no significant modification of LH and FSH occurred after either drug, suggesting that the menstrual cycle disturbances observed during chronic treatment with tiapride might be related to hyperprolactinaemia or be the result of a mechanism similar to that inducing hyperprolactinaemia.

As reviewed by De Wied (1967), psychotropic drugs and especially chlorpromazine interfere with reproductive processes. Many of these psychotropic drugs were found to stimulate prolactin (PRL) secretion (Sultan 1970; Turkington 1972; Beumont et al. 1974): this common property might be the basis of their potential to alter reproduction. Indeed, an inverse relationship between prolactin secretion and gonadal function has long been suspected (L’Hermite 1973).

Justin-Besanson et al. (1974) introduced recently two new substituted benzamides: tiapride or N (diethylaminoethyl) 2 methoxy - 5 sulfamoyl - benzamide, and sulitopride or N [(1 ethyl 2 pyrrolidinyl) methyl] 2 methoxy - 5 ethylsulfonyl - benzamide. These compounds are closely related to sulpiride, which has been reported to frequently induce galactorrhoea - amenorrhoea (L’Hermite et al. 1972a) and systematically hyperprolactinaemia (Delvoye et al. 1974). In the rat, Tuchmann-Duplessis & Mercier-Parot (1976) have recently reported that chronic administration of sulitopride did induce the following modifications: permanent dioestrus, mammary gland development but without lactogenesis, obvious degranulation of pituitary carminophil cells and a possible inhibitory influence on growth hormone secretion.

The present study was initiated to check whether these new compounds would exhibit in the human any acute effects on prolactin and gonadotrophin secretions. Furthermore, one of the two compounds (tiapride) has been tested in female rats both in vitro and in vivo with respect to its ability to modify prolactin secretion and especially to block the direct inhibitory influence of dopamine on the lactotropes of the pituitary gland.

METHODS

In the human

Tests. – Six normal men (21–29 years old) were submitted to an im injection of either 100 mg sulitopride (n=3) or 200 mg tiapride (n=3). Similarly, six normally menstruating women (18–25 years old) received in the luteal phase an im injection of either 100 mg sulitopride (n=3) or 200 mg tiapride (n=3). All tests were started between 08.00 and 09.00 by the insertion of a venous indwelling catheter; blood samples were frequently collected for one h before drug injection and for six h thereafter.

Assays. – All samples were assayed for LH, FSH and PRL by double-antibody radioimmunoassays using non-equilibrium conditions, as previously described (L’Hermite
& Midgley 1971; Robyn et al. 1971). LH and FSH results are expressed as mIU per ml by reference to the 2nd I.R.P. of H.M.G. Prolactin was measured using the homologous human reagents kindly distributed by the N.I.A.M.D.D. (N.I.H., Bethesda, Md., USA) as V.L.S. kit No. 2. A pool of sera collected from women in the immediate post-partum period served as a laboratory standard in each assay: results are expressed in terms of μU per ml of the research standard 71/222 provided by the M.R.C. (Division of Biological Standards and Control, Holly Hill, London, England). Since the distribution of individual values appeared log-normal on the basis of Rankit tests, all statistical analysis were conducted after logarithmic transformation of the data, particularly using variance analysis according to Snedecor & Cochran (1967).

In the rat

Three categories of female Wistar/Furth rats weighing 200–220 g were used:

1) normal, untreated;
2) implanted with the transplantable pituitary tumour (MTW15) into the scapula region 6–8 weeks before use;
3) animals injected sc with tiapride (1 mg/kg body weight) on day 1 at 08.00 and 17.00 and on day 2 at 08.00.

All animals were killed by decapitation at 10.00; the anterior pituitary glands were bisected and incubations of groups of 4 hemipituitary glands with 10 μCi [4,5-3H]-leucine were conducted for 5 h along conditions previously described (MacLeod & Robyn 1977). Aliquots of the incubation medium and of the pituitary homogenates were then submitted to polyacrylamide gel electrophoresis; after staining, the protein bands containing prolactin (as located by subjecting reference prolactin preparations to the same procedure) were submitted to liquid scintillation counting.

The prolactin content in incubation medium and in animal sera was measured by a double-antibody radioimmunoassay utilizing the materials generously supplied by the N.I.A.M.D.D. (N.I.H., Bethesda, Md., USA); results are expressed in terms of the RP-1 rat prolactin preparation.

RESULTS

In the human

Fig. 1 depicts the mean PRL responses in men and women to, respectively, tiapride and sulotriptide.

After tiapride, mean PRL levels increased significantly from 94 or 136 μU/ml at 0 min to maxima of 837 μU/ml (F = 56.1; P < 0.001) or 1359 μU/ml (F = 111.0; P < 0.001) at 30 min for men and women, respectively. This increase in PRL concentrations was statistically significant 5 min after the injection for men (F = 15.1; P < 0.001) as well as for women (F = 52.0; P < 0.001), and mean PRL levels were still higher than control 6 h after injection (F = 15.1; P < 0.001 and F = 87.1; P < 0.001 for men and women, respectively).

Mean basal PRL levels at 0 min were slightly (but not significantly) greater in women than in men. Similarly, mean maximal PRL levels were slightly but not significantly higher in women than in men, their relative amplitude being
also quite comparable: mean maximal levels were 8.9 times greater in men and 10.0 times greater in women than control levels at 0 min.

After sultopride, mean PRL levels increased significantly from 292 or 292 μU/ml at 0 min to maxima of 1088 μU/ml (F = 132.5; P < 0.001) or 1714 μU/ml (F = 306.1; P < 0.001) at 30 min for respectively men or women. This increase in PRL concentrations was statistically significant 5 min after the injection for men (F = 40.6; P < 0.001) as well as for women (F = 119.2; P < 0.001), and mean PRL levels were still higher than control 6 h after injection (F = 56.1; P < 0.001 and F = 203.1; P < 0.001 for, respectively, men and women).

Mean basal PRL levels at 0 min were exactly similar for women and men. Mean maximal PRL levels were significantly (t = 4.20; P < 0.01) higher in

Mean (± SEM) serum prolactin (PRL; expressed as μU 71/222 per ml) levels in response to the im injection (arrows) of 200 mg tiapride or 100 mg sultopride to different groups of, respectively, 3 normal men (M) and 3 normally menstruating women (F) in the luteal phase.
women than in men, as well as their relative amplitude: mean maximal levels were 3.7 times greater in men and 5.9 times greater in women than control levels at 0 min.

Fig. 2 depicts, in comparison to the combined (men plus women) mean PRL responses, the evolutions of LH and FSH levels before and after tiapride or sulotopride administration. LH as well as FSH levels fluctuated very little after drug injection and none of these variations were statistically significant.

In the rat

Table 1 depicts the effects of the in vivo administration of tiapride on the in vitro secretion of prolactin by incubated pituitary glands of female rats implanted with the MtTW15 tumour, which secretes prolactin. As a consequence of the implantation of this tumour, both the biosynthesis and the secretion of the host pituitary gland is markedly inhibited. The injection of tiapride prior to decapitation significantly overcame the inhibitory effect of the MtTW15 tumour on the host pituitary gland (Table 1).

Table 2 depicts the in vitro effects of tiapride and/or dopamine on prolactin secretion by incubated pituitary glands of normal female rats. Dopamine pro-
Table 1.
Effect of \textit{in vitro} tiapride administration on the synthesis and release of prolactin \textit{in vitro} in normal and MtTW15 tumour-bearing female rats (means ± SEM).

<table>
<thead>
<tr>
<th>Incorporation of [3H]leucine into prolactin (cpm/mg pituitary)</th>
<th>Pituary</th>
<th>Medium</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8310 ± 464</td>
<td>5858 ± 617</td>
<td>14168 ± 1051</td>
</tr>
<tr>
<td>MtTW15</td>
<td>3382 ± 191*</td>
<td>1734 ± 146*</td>
<td>5116 ± 216*</td>
</tr>
<tr>
<td>MtTW15 + tiapride</td>
<td>5241 ± 357**</td>
<td>6100 ± 452**</td>
<td>11341 ± 778**</td>
</tr>
</tbody>
</table>

* $P < 0.01$ vs. control.
** $P < 0.01$ vs. MtTW15.

Produced a 62% inhibition of newly synthesized prolactin released in the incubation medium, while tiapride by itself was devoid of any significant effect. On the other hand, when dopamine was introduced together with tiapride into the incubation medium, tiapride significantly overcame the inhibitory effect of dopamine on prolactin secretion.

Table 2.
\textit{In vitro} effects of tiapride and/or dopamine (DA) on prolactin secretion by incubated pituitary glands of normal female rats.

<table>
<thead>
<tr>
<th>Incorporation of [3H]leucine into prolactin (cpm/mg pituitary)</th>
<th>Pituary</th>
<th>Medium</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8001 ± 319</td>
<td>6053 ± 349</td>
<td>14050 ± 569</td>
</tr>
<tr>
<td>DA (5.10^{-7} M)</td>
<td>10780 ± 917</td>
<td>2287 ± 710*</td>
<td>13067 ± 1149</td>
</tr>
<tr>
<td>Tiapride (5.10^{-7} M)</td>
<td>8268 ± 643</td>
<td>7064 ± 811</td>
<td>15332 ± 812</td>
</tr>
<tr>
<td>DA + tiapride</td>
<td>9399 ± 600</td>
<td>5141 ± 370**</td>
<td>14928 ± 1741</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prolactin in medium, as measured by RIA (µg/ml pituitary)</th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.61 ± 0.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA (5.10^{-7} M)</td>
<td>3.54 ± 0.64*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tiapride (5.10^{-7} M)</td>
<td>6.65 ± 0.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA + tiapride</td>
<td>6.69 ± 0.24**</td>
<td></td>
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</tr>
</tbody>
</table>

RIA = radioimmunoassay.
* $P < 0.01$ vs. control.
** $P < 0.01$ vs. DA.
DISCUSSION

As previously shown (MacLeod 1969; MacLeod & Lehmeyer 1974), dopamine inhibited in vitro the prolactin secretion by incubated pituitary glands of normal female rats. While tiapride – at the concentration used – failed to exert any effect on the prolactin secretion by such incubated glands, tiapride significantly overcame and reverted the in vitro inhibitory effect of dopamine.

As previously demonstrated (MacLeod & Abad 1968; MacLeod & Lehmeyer 1973), the implantation of rats with the pituitary tumour MtTW15, which secretes prolactin, induced a significant decrease of the in vitro biosynthesis and secretion of prolactin by the host gland. Prior injection of these tumour-bearing animals with tiapride significantly blocked this inhibitory effect.

Our data demonstrate thus that tiapride antagonizes in vitro the inhibitory activity that dopamine exerts directly on the lactotropes of the rat pituitary gland. In this respect, tiapride appears thus to be comparable to its related compound, sulpiride (MacLeod & Robyn 1977).

The present study demonstrates also the in vivo stimulatory effect of both tiapride and sulprofide on prolactin secretion in the human. After im injection of either psychotropic drug, PRL concentrations increased already by five min and remained elevated for at least six h: this pattern is also quite similar to that observed with the related drug sulpiride (L’Hermite et al. 1978) but contrasted to some extent with those observed after either TRH (thyrotrophin-releasing hormone) or chlorpromazine. The rapidity of the PRL release is quite comparable to that observed after iv injection of TRH, maximal levels being usually reached by 30 min (L’Hermite et al. 1972b) while chlorpromazine action is slower, maximal levels being usually reached by 1–3 h (Frantz et al. 1972).

The long-lasting effect of tiapride and sulprofide, in contrast to TRH, might be attributable to their very different clearance half-lifes from circulation: TRH is indeed being very rapidly inactivated by human serum (Bassiri & Utiger 1972).

A sex difference concerning the absolute magnitude of the PRL release in men and women was clearly evidenced after sulprofide and suggested after tiapride. This finding is in agreement with the concept that, due to their endogenous oestrogenic secretion, women would release more prolactin than men in response to various stimuli such as for example stress (Noel et al. 1972) and TRH (Jacobs et al. 1973).

Acute administration of either drug did not influence at all LH and FSH secretions over the six h period studied. Therefore, the alterations of the menstrual cycle which occurred during chronic administration of tiapride (L’Hermite et al. 1977) might be attributable to the resulting hyperprolactinaemia. An alternate hypothesis would be that tiapride might act at hypothalamic or higher centres in a manner (blockage of dopamine receptors?) similar to that by which
hyperprolactinaemia itself might impair the function of the hypothalamo-pituitary-gonadal axis. Although our data clearly demonstrate that tiapride would promote prolactin secretion directly at the pituitary level, an additional effect at the hypothalamic or higher level can not be excluded. Finally, neuroleptics which interfere with reproductive processes (De Wied 1967) appear to share the common property of stimulating prolactin, this property being itself probably related to their common dopamine receptor antagonistic activity (Seeman et al. 1976; Calabro & MacLeod 1978).

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REFERENCES


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