Abstract. Prolactin was measured in the plasma of neonatal rats after iv and ip injection of the dopamine receptor blocking drug sulpiride, and after its ip injection to neonatal rats’ nursing mothers. The sulpiride dose PRL response relationship in 10–25 day old neonatal rats was similar to that found in lactating rats, with a threshold sensitivity around 29 nmol sulpiride/kg body weight and a maximal response at about 2.9 μmol/kg. Absolute levels of PRL in the neonate (both peak and increment-over-basal) were, however, 90% lower than in adults. Treatment of lactating mothers with a maximally stimulatory dose of sulpiride (2.9 μmol/kg) twice daily for 4 days resulted in small but highly significant increases in neonatal PRL on days 1 and 2 but complete loss of response by day 4. These data demonstrate that there is a close similarity between the responses of maternal and neonatal rats to sulpiride and that transfer of the drug to the neonate via milk can induce neonatal hyperprolactinaemia. The subsequent loss of the neonatal PRL response on chronic exposure to sulpiride may indicate a degree of disturbance of hypothalamic dopaminergic mechanisms. In the clinical situation this would suggest that doses of dopamine receptor-blocking drugs used to enhance maternal milk production should be carefully chosen.

The major neuroregulatory hormone controlling PRL secretion and therefore determining the intensity of hormone stimulation of milk production is dopamine (DA) (Neill 1980). Drugs which interfere in the binding of DA to its receptor (DAR) on the lactotrope enhance PRL secretion (McLeod & Lehmeyer 1974). There are a large number of drugs which act in this way among which are the antiemetic and neuroleptic drugs domperidone, metoclopramide, and sulpiride. Because of their design they bind strongly to the DAR and are therefore long-acting, inducing hyperprolactinaemia which, depending upon the dose, may last from a few, to many hours (L’Hermitte et al. 1978). Hyperprolactinaemia and galactorrhoea are, in relation to the drugs’ primary role, undesirable adverse reactions but their recognition led to the use of such drugs for the improvement of inadequate lactation. Many studies illustrating their effectiveness in this application have been published including those using sulpiride (Assus 1970; Aono et al. 1979; Fenichel et al. 1982; Polatti 1982; Ylikorkala et al. 1982), metoclopramide (Sousa 1975; Lewis et al. 1980; Kauppila et al. 1981), and domperidone (Hofmeyer & van Iddekinge 1983). Many mothers fail to establish successful breast feeding and inadequate milk production is the commonest reason for this failure (Martin & Monk 1982). The delivery of a premature infant may necessitate separation of mother and baby, preventing the normal suckling stimulus or an adoptive mother may wish to start breast feeding. In such instances, an acceptable pharmacological method of initiating, increasing, and maintaining PRL secretion would be of great value. It has been recognized, however, that drugs with neuroleptic properties are powerful agents and the safety of the infant must be an overriding consideration. Proponents of the use of sulpiride for this purpose, having shown its effectiveness in enhancing milk production, suggested that investigations of its
effects on offspring should be carried out (Aono et al. 1979; Kauppila et al. 1981; Ylikorkala et al. 1982). To date, no such work has been published. It has in fact been assumed that the amounts of sulpiride (Aono et al. 1979), domperidone (Hofmeyr et al. 1985), and metoclopramide (Lewis et al. 1980) transferred in milk are too small to have significant clinical effects. Our recent studies on the dose response relationship between sulpiride and human PRL secretion have, however, emphasized the extreme sensitivity of the lactotrope to dopamine receptor blockade (McMurdó et al. 1985).

In the current studies, we have used an animal model to examine the hypothesis that sulpiride, transferred via milk, may induce chronic hyperprolactinaemia in offspring, and having compared the sulpiride dose/PRL response relationship in lactating and neonatal rats have demonstrated the neonatal PRL response to chronic maternal treatment with sulpiride.

Materials and Methods

Animals

Wistar strain rats, raised in our own colony from stock obtained from Charles River Laboratories were mated at between 8 and 10 weeks old. All experiments were carried out on primiparous mothers and their litters.

Administration of sulpiride

Sulpiride (Sigma Chemicals), mol wt 341.4, was dissolved in 100 mmol/l H2SO4 at a concentration of 2.9 mmol/l and diluted in 150 mmol/l saline prior to injection either ip or iv. In those animals injected iv and venipunctured, the entire experiment was performed under ether anaesthesia, injecting and sampling via the external jugular vein.

Prolactin assay

Heparinized blood samples were obtained from venipunctured (see Results) or decapitated pups. Plasma was stored at −20°C prior to assay. Reagents obtained from Dr A. Parlow, NIADDK, were used in a double antibody assay with PEG-assisted separation. Samples were assayed in triplicate, 20 µl/tube. Buffer (10 mmol/l PBS containing 0.1% BSA, 300 µl), plasma or PRL standard diluted in buffer (200 µl), 125I-PRL (10–14 µCi/µg, 10 000 cpm in 100 µl buffer), and rabbit anti PRL (tube dilution 1:15 500 in buffer with 10 mmol/l EDTA, 200 µl) were added in sequence and incubated 20 h at 20°C. Donkey anti-rabbit IgG (1:30) and normal rabbit serum (1:750) obtained from Scottish Antibody Production Unit, Lanarkshire, UK, were added in EDTA buffer (200 µl) and followed by 750 µl 10% PEG 6000 in buffer. The tubes were centrifuged and aspirated 30 min later. The sensitivity of the assay was 40 pg PRL (RP-3) per tube. Coefficients of variation were: intra-assay < 7.5%; inter-assay 12.5%.

Data handling

To equalize variance PRL values were log10 transformed (Selmanoff & Wise 1981) before calculation of mean response parameters. Group comparisons were made by Wilcoxon’s rank sum test (Milton & Tsokos 1983).

![Graph showing PRL concentration over time](image-url)
Results

The sulpiride dose/PRL response relationship in neonatal rats

1) Pubs aged 7 and 18 days. Fifty-six 7-day-old rats and 44 18-day-old rats were separated from their mothers and assigned to one of 7 treatment groups. The treatments were either killing at 0 min or injection with sulpiride (88 nmol, 880 nmol or 8.8 µmol/kg) ip at 0 min and killing at either +5 or +10 min. Approximately equal numbers of male and female pups were in each treatment group and the numbers of animals per group ranged from 7 to 9 among the 7-day-old animals and 6 to 7 among the 18-day-old animals. The PRL concentrations in each treatment group is shown in Fig. 1. Only the principal statistical comparisons are indicated.

The 0 min PRL levels in 18-day-old pups were significantly higher ($P < 0.005$) than those of the 7-day-old pups. With a dose of 88 nmol/kg, PRL responses at 5 min were not significantly higher than normal at either age, but by 10 min mean levels were higher at both 7 days ($P < 0.025$) and 18 days. At a sulpiride dose of 880 nmol/kg 7-day-old pups showed significant PRL increases at both 5 min ($P < 0.025$) and 10 min ($P < 0.005$) whereas the 18-day-old pups only showed a significant increase at 10 min ($P < 0.01$). With the dose of 8.8 µmol/kg both age groups showed significant PRL increases at both 5 and 10 min (7 days, $P < 0.005$; 18 days, $P < 0.01$). The responses of
the 7-day-old pups were more consistent than those of the 18-day-old pups, although their incremental changes were considerably smaller.

2) Pups aged 10–13 and 21–25 days. Fifty-four pups 10–13 days old and 42 pups 21–25 days old were separated from their mothers and assigned to one of 7 treatment groups. The treatments were ip injections of either saline or sulpiride at doses of 29, 88, 290, 880 nmol/kg. 2.9 or 8.8 µmol/kg. All animals were killed 10 min after injection. Approximately equal numbers of male and female pups were in each treatment group, and there were between 7 and 9 animals per group (10–13 days) and 6 animals per group (21–25 days). The PRL concentrations in each treatment group at each age are shown in Fig. 2. At 10–13 days there was a significant (P < 0.05) response to a sulpiride dose of 29 nmol/kg. Between 21 and 25 days a significant increase (P < 0.01) was produced with a dose of 88 nmol/kg. The maximal response was reached with doses of 2.9 µmol/kg or above. Once again, as in the comparison of 7- and 18-day-old animals, the older group (21–25 days) gave less consistent results than the younger group (10–13 days).

Comparison of responses to ip and iv routes for administration of sulpiride

The PRL response of etherised pups to ip or iv sulpiride (880 nmol/kg) and the effect of ether itself was compared in 3 groups of 4 15-day-old pups. Immediately after the induction of light anaesthesia, a small skin incision was made and a blood sample (250 µl) was taken from the external jugular vein. Sulpiride (ip or iv) or saline (iv) was injected and after 5 and 10 min further samples were taken from the re-anaesthetized pups. The PRL levels measured are shown in Fig. 3. There was a small transient rise in PRL 5 min after ether exposure and saline treatment. Sulpiride produced a rapid and sustained rise in PRL and no difference was detectable in the rate or extent of the rise which followed ip or iv administration.

The neonatal PRL response to maternal treatment with sulpiride

The pups from 13 litters, a total of 117 aged between 9 and 17 days at the start of the treatment programme, were used in the study. Four complete litters and 2 half-litters, a total of 43 pups, were used for starting control samples and saline responses. Seven complete litters and 2 half-litters, 74 pups, were used for responses to sulpiride. Pups were killed in half-litter batches, one or two batches per time point, and the stage at which each mother’s 2 half-litters were killed was randomised through the length of the study. After 5 half-litters were killed on the morning of day 1, the control mothers were injected ip with saline at 0, 8, 24, 32, 48 and 72 h and the sulpiride-treated mothers were injected with a dose of 2.9 µmol/kg at the same time intervals. Half-litters from saline and sulpiride-treated mothers were killed 8, 24, 32, 72 and 80 h, and 8, 24, 32, 48, 56, 72 and 80 h, respectively, after treatment began. Prolactin was measured in all plasma samples and the mean carcase weight, after decapitation and collection of trunk blood
PRL concentrations (mean ± 1SE) in the pups of lactating rats injected with saline (○) or sulpiride (●) (2.9 µmol/kg) at 0, 8, 24, 32, 48, 56 and 72 h. The statistical significance of the differences between points indicated is shown by:
- no significant difference, *P < 0.05, **P < 0.025, ***P < 0.005 (Wilcoxon's rank sum test). ▼ injection of saline or sulpiride.

Fig. 4.

was determined for each half-litter killed. The PRL concentrations found at each time interval are shown in Fig. 4. Carcase weights did not differ detectably between the pups of control and sulpiride-treated mothers rising from 13 g at 0 h to 20 g (control) and 20.5 g (sulpiride-treated) after 80 h. Fig. 4 shows that pup PRL was significantly raised 8 h after the start of maternal sulpiride treatment. Samples taken 16 h after the second dose, immediately before the third dose, did not have raised PRL, but 8 h later pups from sulpiride-treated groups had increased PRL. The rise was not as great as on the first day, however. By day 3, the apparent rise in pup PRL 8 h after sulpiride treatment did not reach a significant level. By day 4, although basal PRL had not changed significantly from day 1, there was no PRL response in the pups to the maternal sulpiride dose.

Discussion

It has been established that DAr-blocking drugs can be used to stimulate PRL secretion and enhance milk production in women with inadequate lactation (see Introduction). The drug most widely used for this purpose has been the substituted benzamide sulpiride. Because it is known to be excreted unaltered in breast milk (Aono et al. 1979), the drug has a potential for inducing DAr blockade in the offspring. Sulpiride concentrations have been measured in the breast milk mothers receiving repeated twice daily doses of 145 µmol (50 mg) sulpiride (Aono et al. 1979; Polatti 1982). The data from these chronic studies indicates a stable excretion rate of between 2.2 and 2.9 µmol sulpiride/l milk. Translated into amounts of sulpiride ingested, this represents about 600 nmol to 3 µmol per day depending on the age of the baby. In none of these studies have side effects of the drug been reported to affect the baby. Drug doses of less than 2.9 µmol per 24 h have been regarded as insignificant in terms of the baby's endocrinology. To our knowledge, dopaminergic control of PRL secretion in neonates has not previously been investigated and we have found no data on sensitivity of the neonatal lactotrope to DAr blockade. The effect of 2.9 µmol of
sulpiride on the neonate is therefore difficult to predict. However, we have recently shown that in the adult human female, 2.9 µmol of sulpiride evokes a significant, though transient, increase in PRL secretion (McMurdo et al. 1985). The human infant secretes large amounts of PRL in the early neonatal period and concentrations in plasma fall to normal adult levels within a few months of birth (Guyda & Friesen 1973) suggesting maturation of the neuroregulatory control system during that period.

In the studies reported here we have demonstrated the dose responsiveness of PRL to sulpiride treatment in the neonatal rat. Intraperitoneal injections of sulpiride were used because of the impracticality of chronic venous cannulation of neonatal rats as we have used before with adult animals (Lewis & Howie 1985) and this approach was validated by studies with anaesthetised animals which showed that a moderate dose of sulpiride was equally effective when given ip or iv. Comparing the sulpiride doses for threshold and maximal PRL secretion in neonatal rats, determined in this study, with those of adult rats found in our earlier study (Lewis & Howie 1985), it appears that neonatal and lactating female rats have very similar dose response characteristics. The slightly higher dose threshold observed in 21–25 day old rats may be accounted for by the greater susceptibility of animals of this age to the stress of the experiment, and corresponding increase in variability of results. Furthermore, related to body weight, sensitivity to sulpiride is similar in human adults, lactating rats, and neonatal rats. A sulpiride dose of 145 µmol given to a 50-kg woman induces maximal PRL release (McMurdo et al. 1985) as does 725 nmol sulpiride in a 250 g adult rat, or 58 nmol in a 20 g rat pup. The comparability of human and rat responses to sulpiride suggests that, in endocrine terms, the lactating rat is a suitable model to investigate effects of neonatal exposure to sulpiride. From the age of 7 days, the earliest so far studied, until weaning, the rat's sensitivity to sulpiride, indicated by the dose for half maximal response, did not change appreciably. Variability in the responses found in different animals in the same treatment group increased with the age of the rats, as they become more susceptible to the stress of the experimental situation - handling, removal from the mother and ip injection. The dose response data shown in Fig. 2 probably overestimate considerably the minimum effective dose of sulpiride stimulating prolactin secretion in neonates. More consistent data were obtained in the chronic treatment study in which the family groups were left undisturbed between maternal injections and neonatal sampling times. In that part of the study we treated lactating rats with a maximally stimulatory dose of sulpiride (2.9 µmol/kg) which, based on bioavailability was equivalent to two or three times the usual oral clinical dose of 145 µmol in a woman, and showed that stimulation of their suckling neonates PRL secretion occurred. Although the absolute levels of PRL found in the neonates 8 h after the start of the maternal treatment programme were low on the response range for neonatal animals, they were nevertheless very significantly different from levels found in the pups of placebo-treated mothers, showing that transfer of physiologically significant amounts of sulpiride had taken place via milk. The effect of this chronic intake of sulpiride diminished as maternal treatment continued, suggesting that there had been an upward resetting of the neonate's tonic level of dopaminergic inhibition of PRL, so that by the seventh maternal dose, the small amount of sulpiride reaching the neonates' lactotrope was no longer sufficient to antagonize the effect of the DA released from the median eminence and carried to the pituitary via the portal blood system. We have observed a similar loss of responsiveness when attempting to establish chronic hyperprolactinaemia in adult rats using sulpiride released from osmotic minipumps (Lewis, unpublished). In those experiments as well, normal levels of PRL were restored after an initial 48-h period of hyperprolactinaemia. It has been shown that systemic injections of PRL selectively increase DA turnover in the tuberoinfundibular tract (Moore & Johnston 1982) and therefore the development of tolerance to chronic intake of very low sulpiride doses can be interpreted as a benign homeostatic response. However, in all other hypothalamic dopaminergic tracts, DAR antagonists, including sulpiride, increase DA turnover directly by activating long and short feedback loops associated with pre- and post-synaptic DA receptors (Anden et al. 1966; Tagliamonte et al. 1975; Nowycky & Roth 1978; Moore & Wuerthele 1979). The generalized disturbance of DA-dependent neuroendocrine pathways induced in this way could eventually affect gonadotropin secretion in relation to which hypo-
thalamic DA has both stimulatory and inhibitory functions (McKenzie et al. 1984).

We have not treated lactating rats with lower doses of sulpiride but it is to be expected that the effects on neonatal PRL we have seen are dose-dependent, and a lower maternal dose would induce less stimulation of neonatal PRL secretion and take longer to produce the reflex enhancement of DA turnover. Considerable caution is required in relating these data directly to the lactating woman and her baby. However, by inference it might be proposed that a woman suffering from inadequate lactation might take less than 290 μmol (100 mg) sulpiride each day, doses which, while remaining highly stimulatory to PRL secretion, would give the offspring a lower sulpiride load. Fenichel et al. (1982) showed that 9 μmol of sulpiride per day was a sufficient dose to maintain high PRL levels, although they did not study its effect on milk production or growth of the baby. We are currently using the animal model to study other strategies for reducing the drug intake of the neonate such as preventing suckling for a period of hours later drug administration. Further investigation of PRL release in the offspring of mothers receiving DA-re-blocking treatment is warranted and planned, in order to evaluate the endocrine effects of such compounds in these infants.

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