Br. J. clin. Pharmac. (1989), **28**, 435–441

A dose rising study of the safety and effects on serum prolactin of SK&F 101468, a novel dopamine D_2-receptor agonist

G. ACTON & C. BROOM
Clinical Pharmacology Unit, Smith Kline & French Research Ltd, The Frythe, Welwyn, Hertfordshire AL6 9AR

1 SK&F 101468, a non phenolic indolone derivative, has been characterised preclinically as a novel, potent and specific dopamine D_2-receptor agonist.
2 Its tolerability and effects on serum prolactin were investigated in 14 healthy male volunteers in a study of the first administration of SK&F 101468 to man.
3 Doses between 80 μg and 2.5 mg caused statistically significant ($P < 0.05$) lowering of basal and food stimulated serum prolactin, relative to placebo, over a 6 h post treatment period.
4 SK&F 101468 was well tolerated up to 1 mg with symptoms of nausea and postural hypotension at higher doses.

Keywords SK&F 101468 dopamine agonist prolactin

Introduction

Dopamine receptors may be classified into at least two subtypes (Stoof & Kebabian, 1984). Dopamine D_1-receptors are linked to adenylate cyclase and agonist stimulation leads to increased cyclic AMP formation whilst dopamine D_2-receptors are either independent of, or negatively linked to adenylate cyclase (Schaus & Clemens, 1985). Within the central nervous system dopamine D_2-receptors are located, amongst other places, on the lactotrophs of the anterior pituitary and also postsynaptically within the corpus striatum. In the former location, dopamine has a clear role in the inhibition of prolactin release (Weiner & Ganong, 1978) whilst degeneration of the dopaminergic nigro-striatal pathway is associated with Parkinson's disease (Birkmayer & Hornykiewicz, 1961; Olson *et al.*, 1973).

Following the work of Schelesnyak who first demonstrated a prolactin lowering effect of ergot alkaloids in rats, (Schelesnyak, 1954, 1958), a large number of drugs with dopaminergic D_2-receptor activity have been investigated in the treatment of hyperprolactinaemia and Parkinson's disease. Most information to date exists on the two ergot alkaloids bromocriptine (Parkes, 1977; Thorner *et al.*, 1980), which has now been in clinical use for over 20 years, and pergolide (Franks *et al.*, 1983; Tanner *et al.*, 1986), both of which lack specificity for the dopamine D_2-receptor. Bromocriptine has affinity for both α_1- and α_2-adrenoceptors (Gibson & Samini, 1974; McPherson & Beart, 1983) and also 5-HT receptors (Closse *et al.*, 1984). Pergolide has affinity for both α_1- and α_2-adrenoceptors (McPherson & Beart, 1983). Much research has been aimed, in recent years, at developing more specific dopamine D_2-receptor agonists in the hope that such compounds would be both more potent than currently available drugs and also have a lower incidence of side effects.

SK&F 101468 (4-[2-(dipropylamino)ethyl]-1, 3-dihydro-2H-indol-2-one, monochloride) is a recently developed non-phenolic indolone derivative which has been characterized as a dopamine D_2-receptor agonist (Gallagher *et al.*, 1985). *In vitro* binding studies have further shown SK&F 101468 to be a specific dopaminergic agonist with no significant activity at other receptor types (SK&F: Internal reports). We now extend these findings of D_2-receptor agonism by reporting the safety and effects on serum prolactin of SK&F 101468 in healthy male volunteers in a study of its first administration to man. These results have been presented in preliminary form previously (Broom *et al.*, 1988).

Methods

Fourteen healthy male volunteers, mean age 29 years (range 19–42 years) and mean weight 70.2 kg (range 58–83 kg), participated in this placebo controlled, dose rising study. Written informed consent was obtained from each subject and the study received the approval of a local independent ethics committee. Subjects received up to three doses of SK&F 101468, each on a separate occasion, in order of increasing dose with a randomly positioned placebo day. Study days were separated by at least 48 h.

On each study day, subjects fasted from the previous midnight. Baseline blood samples were taken for subsequent assay of serum prolactin. Measurements of pulse rate and blood pressure were made after a period of supine rest and the appropriate volume of SK&F 101468 solution was then administered orally. Post treatment, pulse rate and blood pressure were recorded at 15 min intervals for 4 h with an ECG lead II trace every 30 min. A light standard lunch was provided at 4 h. At 6 h, pulse rate and blood pressure measurements were made before and after standing and subjects subsequently became ambulant.

Blood samples were taken for measurement of serum prolactin concentrations at the following times post treatment: 30, 60, 90, 120, 150, 180 and 240 min and 6, 8 and 24 h. Serum prolactin was assayed overnight following each study day with a commercially available radioimmunoassay kit (Serono Diagnostics, Code No: 10803). Internal standards ranging from 65–4875 μiu ml^{-1}, calibrated against the WHO 75/504 standard, were employed. The overall coefficient of variation for interassay variability was 8.8%. The sensitivity of the assay was determined as 11 μiu ml^{-1}.

Laboratory safety screening (haematology, biochemistry and urinalysis) were performed before and after each study and also within 1 week of the completion of the study.

Data analysis

The area under the serum prolactin concentration time curve (AUC) was calculated, using the trapezoidal rule, for the following time periods post treatment: 0–4 h, 4–6 h, 0–24 h. AUCs were divided by the relevant time zones to obtain mean values expressed in the original units of measurement.

The areas under the systolic blood pressure, diastolic blood pressure and pulse rate time curves were calculated, using the trapezoidal rule, for the period 0–6 h post treatment and the AUCs divided by the time zone to obtain mean values expressed in the original units of measurement.

For serum prolactin and for the three cardiovascular parameters, the data for the various time periods were analysed, by an analysis of variance model, using GENSTAT 5 software (Numerical Algorithms Group Ltd, Oxford) to fit a linear model with effects for subjects, study periods and treatments. As all doses were not present in all subjects this resulted in a non-orthogonal analysis of variance.

Results are presented in terms of 95% confidence intervals for the difference of each dose of SK&F 101468 from placebo using a pooled estimate of the variance.

As with the simple *t*-test, confidence intervals for the difference of two means can be calculated even when the second mean is based on one observation. However the uncertainty in these estimates is reflected in their (relatively) larger confidence intervals.

Results

Dosage

The doses of SK&F 101468 administered, (and the number of subjects receiving each dose) were: 10 μg (2), 20 μg (2), 40 μg (4), 80 μg (4), 160 μg (5), 320 μg (7), 640 μg (8), 1000 μg (2), 1250 μg (4), 1850 μg (1), 2500 μg (1).

Prolactin data

The mean baseline prolactin over all treatment periods for all subjects was 223 μiu ml^{-1} (s.e. mean 28). Table 1 shows the model derived treatment mean differences from placebo, together with the approximate 95% confidence intervals, for serum prolactin, 0–4, 4–6 and 0–24 h post treatment. Differences from placebo from 0–4 and 4–6 h are shown, respectively, in Figures 1 and 2.

SK&F 101468 produced a statistically significant ($P < 0.05$) lowering of mean serum prolactin, when compared with placebo, in the dose range 80 μg to 2500 μg for the period 0–4 h post treatment. Overall mean differences ranged from -4.4 μiu ml^{-1} (95% CI: -56.5, 47.7) with 10 mcg to -117.4 μiu ml^{-1} (95% CI: -164.8, -70.0) with 1000 μg and mean differences were relatively constant at doses from 320 μg to 2500 μg. For the period 4–6 h after treatment, decreases, relative to placebo, in mean serum prolactin

Table 1 Serum prolactin treatment means, expressed as difference from placebo, together with approximate 95% confidence intervals for that difference

Dose (μg) of SK&F 101468	*Difference from placebo*	*SED*	*Approximate 95% CI Upper*	*Lower*
Prolactin 0–4 h (μiu ml^{-1})				
10	−4.40	25.40	47.72	−56.52
20	−9.50	25.10	42.01	−61.01
40	−19.40	16.50	14.46	−53.26
80	−47.90	15.10	−16.91	−78.89
160	−45.00	14.30	−15.66	−74.34
320	−68.70	12.40	−43.26	−94.14
640	−86.90	12.00	−62.28	−111.52
1000	−117.40	23.10	−70.00	−164.80
1250	−86.20	16.70	−51.93	−120.47
1850	−68.00	30.60	−5.21	−130.79
2500	−77.00	31.00	−13.39	−140.61
Prolactin 4–6 h (μiu ml^{-1})				
10	−3.70	42.80	84.13	−91.5
20	11.40	42.40	98.40	−75.6
40	13.30	27.80	70.35	−43.7
80	−60.10	25.60	−7.57	−112.6
160	−18.40	24.20	31.26	−68.1
320	23.90	20.90	18.99	−66.8
640	−98.20	20.30	−56.54	−139.9
1000	−127.30	39.00	−47.27	−207.3
1250	−121.00	28.10	−63.34	−178.7
1850	−107.80	51.60	−1.92	−213.7
2500	−130.30	52.20	−23.19	−237.4
Prolactin 0–24 h (μiu ml^{-1})				
10	23.10	40.50	106.21	−60.01
20	48.80	40.10	131.09	−33.49
40	11.70	26.30	65.67	−42.27
80	42.30	24.20	91.96	−7.36
160	30.30	22.90	77.29	−16.69
320	0.20	19.80	40.83	−40.43
640	−38.30	19.20	1.10	−77.70
1000	−78.20	36.80	−2.69	−153.71
1250	−24.80	26.60	29.78	−79.38
1850	−128.00	48.80	−27.86	−228.14
2500	−43.40	49.40	57.97	−144.77

were seen at doses between 80 μg and 2500 μg. These differences were statistically significant ($P < 0.05$) except for the 160 and 320 μg doses. Overall differences ranged from 13.3 μiu ml^{-1} (95% CI: −43.7, 70.4) with 40 μg to −130.3 μiu ml^{-1} (95% CI: −237.4, −23.2) with 2500 μg. Mean differences were reasonably constant at doses between 640 μg and 2500 μg.

For the 0–24 h period differences, relative to placebo, were negative for doses of SK&F 101468 of 640 μg and above, with no particular dose response relationship. Only the differences between the 1000 μg and 1850 μg doses and placebo, were statistically significant ($P < 0.05$). Overall differences ranged from 48.8 μiu ml^{-1} (95% CI: −33.5, 131.1) with 20 μg to −128.0 μiu ml^{-1} (95% CI: −228.1, −27.9) with 1850 μg.

Haemodynamic data

There was a statistically significant increase, relative to placebo, in mean pulse rate ranging from 5 to 8 beats min^{-1} following doses between 640 μg and 1850 μg for the 0–6 h period post treatment ($P < 0.05$). Overall differences ranged from −4 beats min^{-1} (95% CI: −9.21, 0.39) with 10 μg to +8 beats min^{-1} (95% CI: 1.86, 13.93) with 1850 μg. There were no statistically significant changes in systolic or diastolic blood pressure relative to placebo.

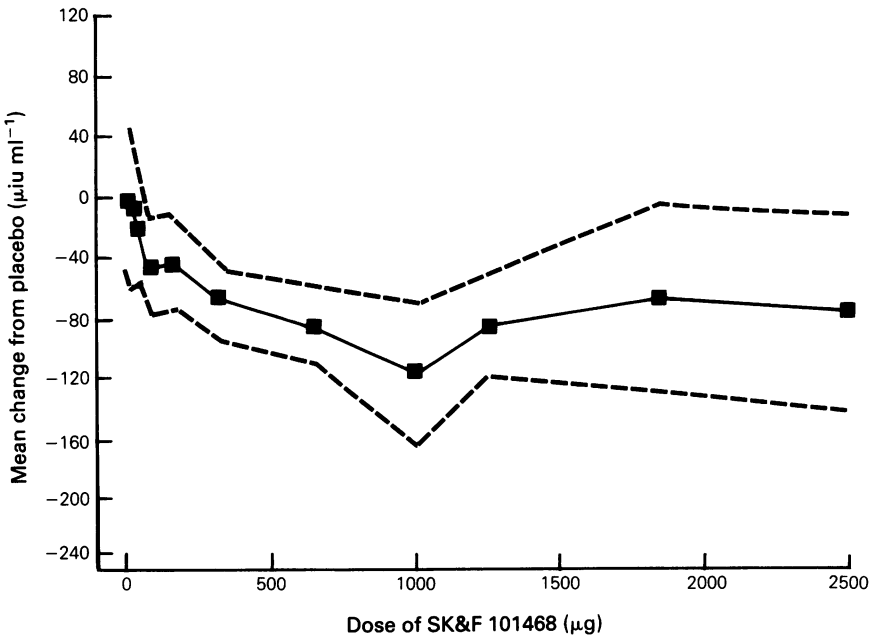

Figure 1 Mean serum prolactin, change from placebo 0–4 h post treatment, and 95% confidence intervals.

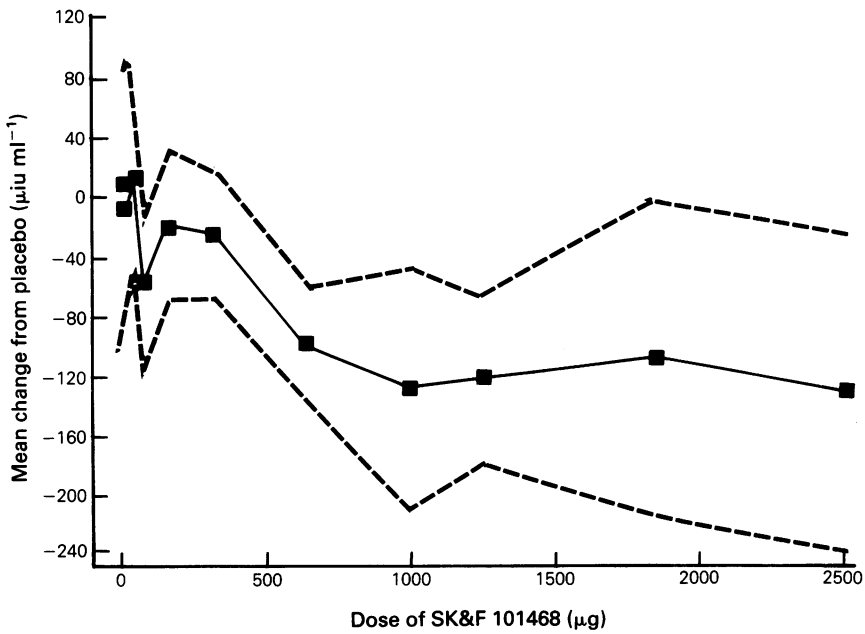

Figure 2 Mean serum prolactin, change from placebo 4–6 h post treatment, and 95% confidence intervals.

Clinical events

Mild, short-lasting, nausea occurred in four subjects at doses of 1250 μg or above, occurring approximately 45–60 min after dosing. Symptoms of postural hypotension occurred in two subjects when standing at 6 h after a 1250 μg dose and both subjects were laid supine before recordings of blood pressure could be made. Neither of two separate subjects who received, respectively, 1850 μg and 2500 μg doses experienced postural hypotension at 6 h.

One subject showed increased frequency of supraventricular ectopic beats on each of two occasions following an 80 μg dose relative to a lower incidence of similar ectopic beats recorded on each of two separate placebo days. The peak incidence of ectopic beats occurred at about 120 min after dosing.

No other events of clinical significance occurred in the study. There were no drug related changes in laboratory safety parameters (haematology, biochemistry or urinalysis).

Discussion

The inhibition of prolactin release by dopamine requires it to bind to the dopaminergic receptors of the anterior pituitary lactotrophs, receptors which will also bind synthetic dopamine agonists. The short plasma half-life of prolactin (approximately 40 min) and its fast metabolic clearance (Cooper *et al.*, 1979) therefore make it a valuable marker for confirming the pharmacological effect of dopamine agonists at D_2-receptors in humans.

In this study, doses of SK&F 101468 between 80 μg and 2500 μg caused marked reductions of circulating levels of basal prolactin. Although the mean 0–4 h prolactin values showed a dose dependent lowering within this range, relative to placebo, the 95% confidence intervals were wide and the prolactin lowering effects of SK&F 101468 may not, in fact, be dose dependent. The effects of SK&F 101468 on basal prolactin are similar to those found with other dopamine agonists which consistently reduce basal levels to low values in healthy volunteers (Van Loon *et al.*, 1979; Lemberger *et al.*, 1980). SK&F 101468 is, however, clearly very potent with an effect evident at doses as low as 80 μg. In general an effect on prolactin was evident within about forty five minutes of dosing which approximates to the serum half life of prolactin (Cooper *et al.*, 1979).

Food acts as a stimulant to prolactin secretion (Quigley *et al.*, 1981; Carlson *et al.*, 1983) and in this study results calculated between 4 and 6 h post treatment, following a standard meal at 4 h, contain a component representing food enhanced prolactin release. SK&F 101468 attenuated this enhanced secretion, relative to placebo, in the same dose range as that effective upon basal prolactin. This is in agreement with the general finding that dopamine agonists attenuate the increase in prolactin which occurs following various other stimuli, such as TRH (Thorner *et al.*, 1978; Perryman *et al.*, 1981) or perphenazine (Lemberger *et al.*, 1974, 1980).

This study was not designed to assess the duration of action of SK&F 101468 on prolactin release. Although 0–24 h mean differences, relative to placebo, were negative for all doses of SK&F 101468 of 640 μg and above, only at two of these dose levels were the differences statistically significant. These results suggest that the effect of higher doses of SK&F 101468 upon serum prolactin may possibly persist over a 24 h period but such an assessment would have required more frequent sampling between the 8 h and 24 h samples. It would therefore be unwise to reach conclusions on the duration of action of SK&F 101468 from the data presented here.

The agonist activity of a compound on the D_2-receptors of the anterior pituitary means that it will also have agonist effects on the D_2-receptors located both presynaptically on post ganglionic sympathetic nerve terminals where receptor activation has a sympatholytic effect (Willems *et al.*, 1985) and within the chemoreceptor trigger zone of the area postrema, and also possibly within the G.I. tract itself (Borison *et al.*, 1974). This explains the predictable liability of D_2-receptor agonists to induce side effects of nausea and postural hypotension.

SK&F 101468 induced nausea at doses between 1250 μg and 2500 μg. In all cases this was mild and although one subject retched, there were no episodes of vomiting. It is interesting, however, that the effects of SK&F 101468 on serum prolactin concentrations occurred at doses as low as 80 μg giving a sixteen fold difference between doses pharmacologically effective on D_2-receptors of the anterior pituitary lactotrophs and doses clinically effective on the D_2-receptors of the chemoreceptor trigger zone. The reasons for this discrepancy are unclear. There is evidence that the dopamine D_2-receptor antagonist, domperidone, can inhibit dopamine agonist induced nausea without affecting dopamine agonist induced lowering of plasma prolactin concentrations, which may be explained by a differential affinity of domperidone for the D_2-receptors of the anterior pituitary lactotrophs and the area postrema (Nappi *et al.*, 1987). Although we know of no evidence for such a clinically relevant

difference in affinity of dopamine agonists for various D_2-receptor binding sites, this remains a possibility in man.

In general SK&F 101468 was well tolerated in this study and was free of some of the side effects reported for other dopamine agonists, such as drowsiness and nasal congestion, which are not readily explicable in terms of D_2-receptor agonism (Lemberger *et al.*, 1980; Rowbotham *et al.*, 1978; Thorner *et al.*, 1978). Such side effects could arise from the activity of these compounds at other receptor sites and the proposed specificity of SK&F 101468 could explain their absence in this study. The occurrence of supraventricular ectopic beats in one subject at a dose of 80 μg is not immediately explicable in terms of the known preclinical pharmacology of SK&F 101468, but ectopic beats have also been reported for pergolide (Lemberger *et al.*, 1980) and L-dopa (Parks *et al.*, 1970). It is interesting that small, although statistically significant, increases in supine pulse rate, relative to placebo, were seen with doses of SK&F 101468 between 640 μg and 1850 μg. Similar small increases in pulse rate have been seen with some other dopamine agonists such as propylbutyldopamine (Fennell *et al.*, 1983) and apomorphine (Rowbotham *et al.*, 1983) but such effects have usually been attributed to activity at postsynaptic dopamine D_1-receptors. The mechanisms underlying the increased pulse rates seen with SK&F 101468 remain unclear.

The anterior pituitary and the chemoreceptor trigger zone both lie outside the blood brain barrier and are pharmacologically peripheral to the central nervous system. This study has therefore demonstrated that SK&F 101468 has potent agonist activity at peripheral D_2-receptors, thus confirming in man the preclinical pharmacology of the compound. SK&F 101468 is currently entering clinical trials in Parkinson's disease to assess its therapeutic utility as a centrally acting D_2-receptor agonist.

We gratefully acknowledge the help of Mr P. Sparrow, Department of Statistical Science, SK&F, Welwyn, who kindly analysed the data.

References

Birkmayer, W. & Hornykiewicz, O. (1961). Der L-Dioxyphenylalanin (L-Dopa) effekt bei der Parkinson-Akinesie. *Wien. klin. Wochenschr.*, **73**, 787–792.

Borison, H. L. (1974). Area postrema: Chemoreceptor trigger zone for vomiting is that all? *Life Sci.*, **14**, 1807–1817.

Broom, C., Acton, G., Gill, C. & Sparrow, P. (1988). Effects of SK&F 101468, a novel dopamine D_2 agonist on supine plasma prolactin and haemodynamics in normal man. *Br. J. clin. Pharmac.*, **26**, 654–655.

Carlson, H. E., Wasser, H. L., Levin, S. R. & Wilkins, J. N. (1983). Prolactin stimulation by meals is related to protein content. *J. clin. Endocrinol. Metab.*, **57**, 334–338.

Closse, A., Frick, W., Dravid, A., Bolliger, G., Hauser, D., Sauter, A. & Tobler, H. J. (1984). Classification of drugs according to receptor binding profiles. *Arch. Pharmac.*, **327**, 95–101.

Cooper, D. S., Ridgway, E. C., Kliman, B., Kjellberg, R. N. & Maloof, F. (1979). Metabolic clearance and production rates of prolactin in man. *J. clin Invest.*, **64**, 1669–1680.

Fenell, W. H., Taylor, A. A., Young, J. B., Brandon, T. A., Ginos, J. Z., Goldberg, L. I. & Mitchell, J. R. (1983). Propylbutyldopamine: haemodynamic effects in conscious dogs, normal human volunteers and patients with heart failure. *Circulation*, **67**, 829–836.

Franks, S., Horrocks, P. M., Lynch, S. S., Butt, W. R. & London, D. R. (1983). Effectiveness of pergolide mesylate in long term treatment of hyperprolactinaemia. *Br. med. J.*, 286, 1177–1179.

Gallagher, G., Lavanchy, P. G., Wilson, J. W., Paul Hieble, J. & De Marinis, R. M. (1985). 4-[2-(Di-n-propylamino)ethyl]-2(3H)-indolone: A prejunctional dopamine receptor agonist. *J. med Chem.*, **28**, 1533–1536.

Gibson, A. & Samini, M. (1979). The effects of bromocriptine on presynaptic and post-synaptic α-adrenoceptors in the muscle vas deferens. *J. Pharm. Pharmac.*, **31**, 826–830.

Lemberger, L., Crabtree, R. & Callaghan, J. T. (1980). Pergolide, a potent long acting dopamine-receptor agonist. *Clin. Pharmac. Ther.*, **27**, 642–651.

Lemberger, L., Crabtree, R., Clemens, J., Dyke, R. W. & Woodburn, R. T. (1974). The inhibitory effect of an ergoline derivative (lergotrile, Compound 83636) on prolactin secretion in man. *J. clin. Endocrinol. Metab.*, **39**, 579–584.

McPherson, G. A. & Beart, P. M. (1983). The selectivity of some ergot derivatives for α_1 and α_2 adrenoceptors of rat cerebral cortex. *Eur. J. Pharmac.*, **91**, 363–369.

Nappi, C., Colace, G., Di Renzo, G. F., Taglialatela, M., Amoroso, S., Annunziato, L. & Montemagno, U. (1987). Domperidone antagonises bromoergocriptine-induced nausea and vomiting without affecting its inhibition of prolactin secretion in puerperal women. *Eur. J. clin. Pharmac.*, **32**, 457–460.

Olson, L., Nystrom, B & Seiger, A. (1973). Monoamine fluorescence histochemistry of human post mortem brain. *Brain Res.*, **63**, 231–247.

Parkes, D. (1977). Bromocriptine. *Adv. Drug. Res.*, **12**, 247–344.

Parks, L. C., Watanabe, A M. & Kopin, I. J. (1970).

Prevention of reversal of L-Dopa-induced cardiac arrhythmias by decarboxylase inhibitors. *Lancet*, **ii**, 1014–1015.

Perryman, R L., Rogol, A. D., Kaiser, D. L., Macleod, R. M. & Thorner, M. O. (1981). Pergolide Mesylate: its effects on circulating anterior pituitary hormones in man. *J. clin. Endocrinol. Metab.*, **53**, 772–778.

Quigley, M. E., Ropert, J. F. & Yen, S. S. C. (1981). Acute prolactin release triggered by feeding. *J. clin. Endocrinol. Metab.*, **52**, 1043–1045.

Rowbotham, M. C., Joseph, M. S., Jones, R. T. & Keil, L. C. (1983). Failure of naloxone to reverse apomorphine effects in humans. *Psychoneuroendocrinology*, **8**, 95–102.

Schaus, J. M. & Clemens, J. A. (1985). Dopamine receptors and dopaminergic agents. *Ann. Reports med. Chem.*, **20**, 41–50.

Schelesnyak, M. C. (1954). Ergotoxin inhibition of deciduoma formation and its reversal by progesterone. *Am. J. Physiol.*, **179**, 304–310.

Schelesnyak, M. C. (1958). Maintenance of gestation in ergotoxine – treated rats by exogenous prolactin. *Acta endocrinologica*, **27**, 99–109.

Stoof, J. C. & Kebabian, J. W. (1984). Two dopamine receptors: biochemistry, physiology and pharmacology. *Life Sci.*, **35**, 2281–2296.

Tanner, C. M., Goetz, C. G., Glantz, R. H. & Klawans, H. L. (1986). Pergolide Mesylate: Four years experience in Parkinson's disease. In *Advances in neurology*, volume 45, eds Yahr, M. D. & Bergmann, K. J., pp 547–549. New York: Raven Press.

Thorner, M. O., Ryan, S. M., Wass, J. A. H., Jones, A., Bouloux, P., Williams, S.& Besser, G. M. (1978). Effect of the dopamine agonist, Lergotrile Mesylate, on circulating anterior pituitary hormones in man. *Endocrinol. Metab.*, **47**, 372–378.

Thorner, M. O., Fluckiger, E. & Calne, D. B. (1980). *Bromocriptine. A clinical and pharmacological review*. New York: Raven Press.

Van Loon, G. R., Sole, M. J., Bain, J. & Ruse, J. L. (1979). Effects of Bromocriptine on plasma catecholamines in normal man. *Neuroendocrinology*, **28**, 425–434.

Weiner, R. I. & Ganong, W. F. (1978). Role of Brain monoamines and histamine in regulation of anterior pituitary secretion. *Physiol. Rev.*, **58**, 905–976.

Willems, J. L., Buylaert, W. A., Lefebvre, R. A. & Bogaert, M. G. (1985). Neuronal dopamine receptors on autonomic ganglia and sympathetic nerves and dopamine receptors in the gastrointestinal tract. *Pharmac. Rev.*, **37**, 165–216.

(Received 16 February 1989, accepted 19 June 1989)