Opiate analgesics: the effect of agonist-antagonist character on prolactin secretion

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Opiate agonist drugs such as morphine are known to stimulate the secretion of pituitary hormones; whether this stimulation represents a direct action on the pituitary gland rather than an indirect action via the hypothalamus is not known (Tolis, Hickey & Guyda, 1975; Bruni, van Vugt & others, 1977; Shaar, Frederickson & others, 1977). In contrast, recent reports have shown that the narcotic antagonist naloxone inhibits prolactin release (Bruni & others, 1977; Shaar & others, 1977). This observation taken together with reports that enkephalins, like morphine, induce prolactin release have led to the suggestion that endogenous opioid peptides may modulate endocrine function in addition to pain perception (Cocchi, Santagostino & others, 1977; Cusan, Dupont & others, 1977; Rivier, Vale & others, 1977; Shaar & others, 1977). Partial agonist analgesics exhibit a mixture of narcotic agonist and antagonist actions. One such drug which has been recently introduced into clinical practice is the oripavine derivative buprenorphine. This drug has been shown to be more potent than morphine as a narcotic agonist but in addition it shows a complex profile as a narcotic antagonist (Cowan, Lewis & Macfarlane, 1977). To determine the effects of partial agonist analgesics on prolactin secretion buprenorphine and cyclazocine have been compared with both opiate agonists and antagonists for their ability to induce prolactin secretion in the rat.

Male Sprague-Dawley rats (160–180 g) were used for the assays. Morphine, buprenorphine and cyclazocine were administered subcutaneously 60 min before blood collection. Enkephalin analogues were administered intravenously 5 min before blood collection. Naloxone was given subcutaneously before analgesic dosing (10 min before enkephalins; 5 min before alkaloids). Blood samples were obtained by cardiac puncture and plasma separated by centrifugation (750 $g \times 20$ min at 4°). Samples were stored at −20° until required for assay. Prolactin was estimated using the NIAMDD rat prolactin radioimmunoassay kit. For *in vitro* experiments anterior pituitary cells were isolated by sequential incubation in 0·25% trypsin solution (Difco) and soya bean trypsin inhibitor (Schrey, Brown & Ekins, 1977). Following mechanical dispersion in calcium and magnesium free Krebs bicarbonate Ringer medium, the cells were suspended in Ham F10 medium containing 0·5% bovine serum albumin. 100 μl aliquots of suspension were incubated with 400 μl samples of drug solution for 60 min at 37°. When incubation was completed the cells were separated by centrifugation (200 $g \times 5$ min) and the prolactin content of the supernatant estimated as before.

As reported by Bruni & others (1977) and Shaar & others (1977) morphine (10 mg kg^{-1}, s.c.) elevated plasma prolactin concentrations when compared with saline-treated controls (Table 1). Previous treatment with naloxone (3 mg kg^{-1}, s.c.) antagonized the stimulation induced by morphine, but interpretation of this result is complicated by the fact that naloxone also lowered basal prolactin concentrations (Table 1). Nevertheless, this latter result may itself be interpreted as indirect evidence for a tonic influence by endogenous opioid on plasma prolactin concentrations. Previous workers have reported that administration of enkephalins provokes the secretion of prolactin *in vivo* but there is controversy as to whether such activity results from a direct action on the anterior pituitary gland (Lien, Fenichel & others, 1976; Cocchi & others, 1977; Shaar & others, 1977; Grandison & Guidotti, 1977; Cusan & others, 1977). Tyr-D-Ala-Gly-Phe-Met NHC_3H_7 (Peptide A) and Tyr-D-Ala-Gly-$NH(CH_2)_2C_6H_5$ (Peptide B) are two enkephalin analogues designed to probe enkephalin structure activity relations and to be resistant to enzymatic degradation. Both *in vitro* and *in vivo* pharmacological tests have shown the peptides to be opiate agonists (Morgan, Bower & others, 1977). Consideration of Table 2 shows that both peptides induced a dose-related rise in plasma prolactin concentrations and that such rises exceeded the equivocal responses produced by the parent peptide methionine-enkephalin. Supplementary experiments demonstrated that, like morphine, the peptide-induced secretion of prolactin was significantly antagonized by prior treatment with naloxone (3 mg kg^{-1}, s.c.; see also Bruni & others, 1977; Shaar & others, 1977).

Table 1. *The effect of morphine and naloxone on the concentration of prolactin in rat plasma.*

Treatment	Plasma prolactin, ng ml^{-1}	*P* value
1. Control	27 ± 8	—
2. Morphine (10 mg kg^{-1}, s.c.)	62 ± 7	<0·01*a*
3. Naloxone (3 mg kg^{-1}, s.c.)+ Morphine (10 mg kg^{-1}, s.c.)	8 ± 3	<0·001*b*
4. Naloxone (3 mg kg^{-1}, s.c.)	6 ± 1	<0·02*a*

a = *P* value compared with control; *b* = *P* value compared with morphine alone treatment.
Values are mean ± s.e.m. from 8 animals.

† Correspondence.

Table 2. *The effect of enkephalins on the concentration of prolactin in rat plasma.*

Treatment	Dose mg kg^{-1}, i.v.	Plasma prolactin ng ml^{-1}	*P vs* control
Control	—	6 ± 1	—
Peptide A	0·1	13 ± 3	<0·05
	1·0	41 ± 9	<0·01
	10·0	57 ± 6	<0·001
Control	—	30 ± 6	—
Peptide B	0·1	43 ± 9	NS
	1·0	56 ± 10	<0·05
	10·0	67 ± 9	<0·01
Control	—	30 ± 6	—
Met-enkephalin	0·1	37 ± 14	NS
	1·0	25 ± 7	NS
	10·0	44 ± 9	NS

Values are mean ± s.e.m. from 7 animals.
Peptide A is Tyr-D-Ala-Gly-Phe-Met NHC_3H_7; Peptide B is Tyr-D-Ala-Gly-$NH(CH_2)_2C_6H_5$.

Table 3. *The effects of buprenorphine and cyclazocine on the concentration of prolactin in rat plasma.*

Treatment	Dose mg kg^{-1}, s.c.	n	Plasma prolactin, ng ml^{-1}	*P vs* control
Control	—	22	16 ± 2	—
Buprenorphine	0·1	16	7 ± 1	<0·001
	1·0	24	6 ± 0·6	<0·001
	10·0	8	7 ± 0·3	<0·001
Control	—	16	9 ± 1	—
Cyclazocine	1·0	16	4 ± 0·4	<0·001
	10·0	8	3 ± 0·3	<0·001

Values are mean ± s.e.m.; n = number of animals.

In contrast to morphine and the enkephalins neither buprenorphine nor cyclazocine elevated prolactin concentrations (Table 3). In fact both drugs produced a decrease similar to that observed after naloxone. Hence buprenorphine and cyclazocine at the doses used acted as antagonists rather than agonists in this endocrine test.

To ascertain whether the elevation of plasma prolactin concentrations induced by morphine and the enkephalins resulted from a direct effect on anterior pituitary cells, these compounds were incubated with anterior pituitary tissue *in vitro*. The results obtained for morphine (5 ng–50 μg) and Peptide A (5 ng–50 μg) did not differ significantly from the control value of 31 ± 3 ng prolactin (values ranged from 25 ± 2 to 45 ±7 ng prolactin) (similar results were obtained for both Peptide B and methionine enkephalin). Thus neither morphine nor Peptide A provoked a release of prolactin, so that the increased concentrations observed *in vivo* appear to result indirectly from an opiate response in the brain. Such a finding agrees with Shaar & others (1977), but is at variance with the original observation of Lien & others (1976).

Thus the present experiments have confirmed that both alkaloid and peptide opiate agonists induce a rise in plasma prolactin concentrations *in vivo* and that such rises are antagonized by prior administration of naloxone. That naloxone also lowered basal prolactin concentrations supports the suggestion that endogenous opioid peptides may be involved in the modulation of prolactin secretion. That the peptides do not have a direct effect on anterior pituitary cells indicates that such modulation occurs in the cns rather than in the pituitary itself. Partial agonist analgesics, as exemplified by buprenorphine and cyclazocine, do not provoke prolactin secretion. Possibly a wider examination of the endocrine effects produced by analgesics may provide a useful aid to their pharmacological characterization.

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