Morphine-induced changes in striatal dopamine mechanisms not evoked from the dopamine nerve terminals

N.-E. ANDÉN*, M. GRABOWSKA-ANDÉN, *Department of Pharmacology, University of Göteborg, Fack, S-400 33 Göteborg, Sweden*

Morphine and morphine-like drugs produce both functional and biochemical effects similar to those seen after neuroleptic drugs (for review, see Kuschinsky, 1976). Thus, opioids induce hypokinesia, catalepsy and muscular rigidity in rats. Furthermore, opioids accelerate the turnover of the brain dopamine, as observed by many different techniques. The modes of action of opioids and neuroleptics are probably not identical, however, since only the effects of the opioids are inhibited by narcotic antagonists, such as naloxone (for review, see Kuschinsky, 1976). Therefore, the opioids probably act on special opiate receptors, whereas the neuroleptics reduce the stimulation of dopamine receptors. In the present investigation, we have studied from where morphine might evoke the above changes.

Male Sprague-Dawley rats, 150–230 g were used. Drugs used were: morphine HCl (Pharmacopoea Nordica), haloperidol (Leo, Helsingborg), apomorphine HCl 1/2 H_2O (Sandoz, Basle), 3-hydroxybenzylhydrazine HCl (NSD 1015; synthesized in this department), naloxone HCl (Endo Lab, Garden City, N.Y.), γ-butyrolactone (Merck, Darmstadt). Haloperidol was dissolved in a few drops of glacial acetic acid and 5·5% glucose was added to the volume. The other drugs were dissolved in 0·9% NaCl. The doses refer to the forms mentioned.

To investigate the importance of the corpus striatum for the changes in muscle tone induced by morphine (30 mg kg^{-1}, i.p.), 1 μl 25% KCl was injected into the corpus striatum on one side and an equimolar amount of NaCl (1 μl 20%) on the opposite side. The injection cannulae were introduced by means of guide cannulae implanted the day before when the rats were anaesthetized and placed in a stereotaxic instrument (Stock, Magnusson & Andén, 1973). The rats were put in plastic cylinders (diameter 50 cm) after the intrastriatal injections and they were observed both for direction of body turning and for the number of rotations per unit time. The rats treated unilaterally with 25% KCl respond to dopamine receptor agonists and antagonists in the same way as after removal of the corpus striatum on the same side, indicating that the 25% KCl inactivates the corpus striatum on the treated side (Stock & others, 1973).

Morphine (30 mg kg^{-1}, i.p., 15 min before the KCl injection, n = 11) did not cause any turning of the rats with the corpus striatum inactivated on one side, despite the presence of a clearcut catalepsy. Haloperidol (2 mg kg^{-1}, i.p., n = 6) given 75–90 min after morphine, did not induce any asymmetry while haloperidol alone (2 mg kg^{-1}, i.p., 15 min before the KCl injection, n = 10) turned the rats markedly to the NaCl-treated side for several hours. Morphine (30 mg kg^{-1}, i.p.) given 75 min after haloperidol (2 mg kg^{-1}, i.p., 15 min before the KCl injection, n = 5) almost completely abolished the haloperidol-induced asymmetry.

In the same experimental model, apomorphine (2 mg kg^{-1}, i.p., immediately after the KCl injection, n = 9) produced a marked circling (147 $\pm$ 10·3 rotations per 45 min, mean $\pm$ s.e.) to the KCl-treated side of rats pretreated with reserpine (10 mg kg^{-1}, i.p., 4 h before the KCl injection). Morphine (30 mg kg^{-1}, i.p., 30 min before the KCl injection, n = 6) completely prevented the apomorphine-induced turning (0 $\pm$ 0·0 rotations per 45 min, mean $\pm$ s.e.). Thus, morphine inhibited the opposite asymmetric behaviours induced by the dopamine receptor antagonist haloperidol and the dopamine receptor agonist apomorphine, indicating that morphine does not act directly on dopamine receptors.

To investigate the effect of morphine on the tyrosine hydroxylase activity *in vivo*, the accumulation of dopa following complete inhibition of the dopa decarboxylase by 3-hydroxybenzylhydrazine (NSD 1015; 100 mg kg^{-1}, i.p., 30 min) was determined in different brain parts (Carlsson, Davis & others, 1972). The rats were decapitated under light chloroform anaesthesia and the brains were quickly removed and dissected on an ice-cooled glass plate under a microscope. The dopamine-rich corpus striatum, the noradrenaline-predominant hemispheres (neocortex plus cerebellum) and the noradrenaline-predominant brain stem (thalamus, hypothalamus, mesencephalon, pons, medulla oblongata) from each rat were collected. After homogenization, cation exchange chromatography and oxidation, dopa was determined spectrofluorimetrically (Kehr, Carlsson & Lindqvist, 1972a). The accumulation of dopa in the dopamine nerve terminals is enhanced both at increased depolarization of the nerve terminals as well as in the absence of nerve impulses (Andén, Magnusson & Stock, 1974). On the other hand, the accumulation of dopa in the noradrenaline nerve terminals increases and decreases when the impulse flow is accelerated and discontinued, respectively (Grabowska & Andén, 1976).

The dopa concentrations after dopa decarboxylase inhibition in the corpus striatum, the hemispheres and the brain stem of rats treated with morphine are presented in Table 1. A significant increase in dopa accumulation was observed in all three regions under the influence of morphine. The effects of morphine were

* Correspondence.

Table 1. *Effects of morphine (30 mg kg^{-1}, i.p., 60 min^{+}), naloxone (10 mg kg^{-1}, i.p., 65 min^{+}) γ-butyrolactone (GBL; 750 mg kg^{-1}, i.p., 35 min^{+}) and apomorphine (2 mg kg^{-1}, i.p., 40 min^{+}) on the dopa accumulation (μg g^{-1}) in the corpus striatum, the hemispheres and the brain stem of rats induced by 3-hydroxybenzylhydrazine (NSD 1015, 100 mg kg^{-1}, i.p., 30 min^{+}).*

Treatment	Corpus striatum Dopa^{++}		Corpus striatum Difference^{+++}	Hemispheres Dopa^{++}		Hemispheres Difference^{+++}	Brain stem Dopa^{++}		Brain stem Difference^{+++}
1 NSD	0·93	(11)		0·058	(10)		0·17	(11)	
2 Morphine + NSD	1·50	(10)	2–1: $P < 0·001$	0·077	(10)	2–1: $P < 0·05$	0·24	(10)	2–1: $P < 0·001$
3 Naloxone + NSD	0·85	(5)	3–1: $P > 0·05$	0·068	(5)	3–1: $P > 0·05$	0·15	(5)	3–1: $P > 0·05$
4 Naloxone + morphine + NSD	0·93	(5)	4–2: $P < 0·001$	0·059	(5)	4–2: $P > 0·05$	0·19	(5)	4–2: $P < 0·01$
5 GBL + NSD	2·87	(8)	5–1: $P < 0·001$	0·102	(8)	5–1: $P < 0·001$	0·19	(8)	5–1: $P > 0·05$
6 Morphine + GBL + NSD	2·61	(5)	6–5: $P > 0·05$	0·073	(5)	6–5: $P < 0·05$	0·17	(5)	6–5: $P > 0·05$
7 Apomorphine + GBL + NSD	0·73	(5)	7–5: $P < 0·001$ 7–1: $P > 0·05$	0·055	(5)	7–5: $P < 0·001$ 7–1: $P > 0·05$	0·14	(5)	7–5: $P < 0·05$ 7–1: $P > 0·05$
8 Morphine + apomorphine + GBL + NSD	0·54	(5)	8–7: $P > 0·05$	0·048	(5)	8–7: $P > 0·05$	0·15	(5)	8–7: $P > 0·05$

$^{+}$ Before death.
$^{++}$ Mean with number of experiments in parentheses.
$^{+++}$ Statistical significance by one-way analysis of variance followed by *t*-test ($F = 57·828$, 4·7621 and 7·7249 for the corpus striatum, the hemispheres and the brain stem, respectively; d.f. within groups = 46, 45 and 46, respectively; variance within groups = 0·0882851, 0·000424404 and 0·00115176, respectively).

blocked by naloxone indicating that they were the result of stimulation of opiate receptors (cf. Garcia-Sevilla, Ahtee & others, 1978).

The morphine-induced increase in the dopa accumulation in the corpus striatum could be due to an increased nerve impulse flow in the nigro-neostriatal dopamine neurons but it could also be due to a direct effect on the dopamine nerve terminals in the corpus striatum. To differentiate between these possibilities, morphine was given to rats treated with the γ-hydroxybutyrate precursor γ-butyrolactone. This compound completely inhibits the normal firing of the dopamine cells in the mesencephalon as well as the accelerated firing produced by neuroleptic drugs (Roth, Walters & Aghajanian, 1973; Walters & Roth, 1976). The interruption of the nerve impulse flow following γ-butyrolactone markedly increased the dopa accumulation in the corpus striatum (Table 1). This effect was completely inhibited by apomorphine. This apomorphine-induced action was probably due to stimulation of dopamine autoreceptors on the dopamine nerve terminals in the corpus striatum, as also seen during cessation of the nerve impulses following axotomy (Kehr, Carlsson & others, 1972b; Roth & others, 1973; Walters & Roth, 1976). Morphine did not affect the γ-butyrolactone-induced increase in dopa accumulation in the corpus striatum. Nor did morphine counteract the apomorphine-evoked inhibition of the dopa accumulation in the corpus striatum, as neuroleptic drugs do (Kehr & others, 1972b; Walters & Roth, 1976), indicating that morphine does not block dopamine autoreceptors. Thus, morphine seems to be dependent on nerve impulses in order to increase the synthesis of dopamine in the corpus striatum. Morphine might enhance the stimulatory effect per nerve impulse by an action on the dopamine nerve terminals. This alternative is not likely since morphine has been reported to inhibit rather than stimulate the release of dopamine from nerve terminals of striatal slices during depolarization by hypertonic KCl (Celsen & Kuschinsky, 1974). On the other hand, systemic administration of morphine increases the firing rate of the dopamine cells in the substantia nigra (Iwatsubo & Clouet, 1977; Nowycky, Walters & Roth, 1978). Therefore, it is likely that morphine stimulates the turnover of dopamine by facilitating the firing of the dopamine neurons. It is not known from where morphine produces this effect. It might be compensatory to the morphine-induced catalepsy, but morphine stimulates the turnover of dopamine also in species not showing catalepsy following morphine (for references, see Kuschinsky, 1976). In any case, the increase in the turnover of the brain dopamine cannot be responsible for the morphine-induced changes in muscle tone. The increase in dopa accumulation in the noradrenaline-predominant areas of the brain following systemic treatment with morphine indicates that morphine accelerates the firing of the noradrenaline neurons since the synthesis of noradrenaline varies proportionally to the depolarization of the noradrenaline nerve terminals (Grabowska & Andén, 1976).

In conclusion, morphine inhibits the striatal functional effects of apomorphine and haloperidol in rats by an action outside the corpus striatum and it stimulates the synthesis of both dopamine and noradrenaline, probably by accelerating the firing of the dopamine and noradrenaline neurons.

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Inhibition of oestrogen-induced increase in uterine blood flow in the rat

S. PHAILY, JUDITH SENIOR*, *School of Studies in Pharmacology, University of Bradford, Bradford BD7 1DP, U.K.*

Studies in the ovariectomized rat have shown that uterine blood flow may be significantly increased by treatment with oestrogen (Spaziani & Suddick, 1967). This response may be modified by pretreatment with mepyramine (a histamine H_1-receptor antagonist), cellulose sulphate (a kininogen depleting agent) and inhibitors of prostaglandin synthesis. However, pretreatment with any of these pharmacological agents never produced more than a 50% inhibition of the oestrogen-induced response (Phaily & Senior, 1978). It appears that the mediators may be acting synergistically to increase the uterine blood flow and this experiment was designed to test the hypothesis.

The methodology and validation of the measurement of uterine blood flow using a labelled microsphere technique have been previously reported (Phaily & Senior, 1978). Mature virgin female rats were used (200–250 g) housed in light and temperature controlled conditions. The rats were bilaterally ovariectomized by the dorsal route at least 14 days before the experiments were made. Oestradiol (oestra-1,3,5(10)-triene-3,17-diol) (BDH, Poole, U.K.) was administered in a 10% (v/v) solution of propylene glycol into the tail vein of the restrained rat 1 h before the blood flow determinations were made. Pretreatment, where used, was as follows: A prostaglandin synthesis inhibitor, AH 7170 (2-*m*-(*p*-chlorobenzoyl) phenyl-propionic acid) (Allen & Hanbury Research Ltd., Ware, U.K.) was dissolved in 10% (w/v) sodium bicarbonate solution and administered orally in a dose of 1 mg kg^{-1} twice daily for two days, the last dose was given 60 min before oestradiol was injected. Cellulose sulphate, prepared by the method of Astrup, Galsmar & Volkert (1944), was given at 1 mg kg^{-1} (i.v.) into the restrained rat, mepyramine maleate (May & Baker, Dagenham, U.K.), was given at 5 mg kg^{-1} (i.p.); both drugs were dissolved in 0·9% (w/v) NaCl and given 60 min before the oestradiol injection.

The results were analysed using Student's *t*-test modified to compare samples having different variances (Snedecor & Cochran, 1967).

Blood flow through the uterus in the ovariectomized rat is low and only represents around 0·07% of the total cardiac output (Table 1). This result is at the lower limit of the estimation procedure. Injection of oestradiol produces an increase in uterine blood flow 1 h after the injection but the rise is almost completely inhibited by

* Correspondence.