Br. J. Pharmac. (1970), **39**, 789–797.

Hyposensitivity to drugs induced by morphine and some related substances in the cat

E. CONTRERAS* AND F. HUIDOBRO

Department of Pharmacology, Catholic University of Chile, Santiago, Chile

Summary

1. Hyposensitivity to noradrenaline occurred concomitantly with morphine tachyphylaxis in the absence of any obvious depressant effect on the heart or peripheral circulation.

2. The actions of adrenaline, noradrenaline and angiotensin and, occasionally, other drugs were tested before and after repeated injections of morphine or one of ten related substances in various preparations of the spinal cat.

3. Tachyphylaxis in response to morphine could be related to a simultaneous decrease in the tissue responses to adrenaline, noradrenaline and angiotensin but such a parallelism has not been shown for the substances related to morphine.

Introduction

It is well known that tolerance develops in respect of some actions during chronic morphine administration but a more acute form of hyposensitivity can also be produced. A tachyphylaxis of this type is observed in the blood pressure of the cat when repeated doses of morphine are given at short intervals (Evans, Nasmyth & Stewart, 1952). In this connexion Huidobro, Huidobro & Lewin (1968) noted that, during the period of morphine hyposensitivity, there was also a diminution in the pressor response to noradrenaline which they suggested might be due to a prolonged cardiac depressant action of morphine. This possibility is now further examined.

Many workers have shown that morphine induces a state of hyposensitivity not only to itself but also to other drugs and to nerve stimulation in a variety of organs: for example, cat superior cervical ganglion (Trendelenburg, 1957); guinea-pig ileum (Kosterlitz & Robinson, 1958). In an attempt to correlate such findings, the development of tachyphylaxis to morphine and ten related substances in a variety of preparations of the cat has been investigated in parallel with any concomitant modification of the responses of these preparations to adrenaline, noradrenaline, angiotensin and, occasionally, other drugs.

Methods

Ninety adult cats (1·8–3·6 kg) were used. Each was anaesthetized with sodium pentobarbitone (33 mg/kg, intraperitoneally), a tracheal cannula was inserted and

* Present address: Department of Pharmacology, I.C.M.B. University of Concepcion, Concepcion, Chile.

artificial respiration applied. Both vagi and the cervical sympathetic nerves were cut and the spinal cord transected at the atlanto-occipital junction. The animal was then given heparin (2,500 units).

Statham transducers (P 23 AC) were used to monitor the carotid blood pressure (1 mmHg≡1·333 mbar), the superior vena caval pressure close to the heart and the left ventricular pressure. The hindquarters of some cats were perfused through the lower end of the aorta using a constant volume pump (range, 18–36 ml/min in different experiments); changes in vascular resistance were measured as variations in perfusion pressure (range, 100–250 mmHg).

Contractions of the tibialis anterior were recorded, after fixing the leg, by attaching the distal tendon of the muscle to a Grass force-displacement transducer (FT 10C). For direct muscle stimulation, one electrode was placed into the distal tendon and the other into the muscle belly; for indirect stimulation, the electrodes were placed on the appropriate motor nerve. In both cases, rectangular maximal stimuli of 0·1 ms duration were delivered every 4 s by a Grass stimulator (S 4). Contractions of the muscle were also produced by drugs which were injected either intravenously or intra-arterially into the distal end of the abdominal aorta after ligation of the median sacral and contralateral common iliac arteries. For the experiments employing direct muscle or drug stimulation, the tibialis anterior had been denervated 4–10 days earlier.

Contractions of the nictitating membrane were recorded, after removal of the ipsilateral eye and superior cervical ganglion, with the animal's head fixed. The activities of some visceral smooth muscles were measured as follows. The distal end of one uterine horn was cut between ligatures and the horn freed from its mesentery; the urinary bladder was emptied and the ureters tied; the small intestine was transected at one point. The free border of the nictitating membrane or the free ends of the other organs were connected to a polygraph via a Grass force-displacement transducer (FT 03C). The visceral preparations were covered with a glass bell jar to minimize drying and loss of temperature.

Drugs

Drugs were dissolved in distilled water and administered via a femoral venous cannula, unless otherwise stated. Dosages are expressed in terms of the free bases. In the majority of experiments to induce hyposensitivity, morphine or its related substance was given as a series of relatively small, but gradually increasing, doses repeated at about 5 min intervals (see legend to Fig. 3) to avoid the intense hypotension, cardiac arrhythmias and spinal convulsions which could be induced by single large doses of some of these substances.

Results

The findings are collated in Table 1 and are here detailed only for morphine and nalorphine. At the end of this section any unusual effects of the other related substances are specified.

Effects of morphine and nalorphine

Cardiovascular actions. When morphine was administered by a similar scheme and in similar dosage to that shown in the legend to Fig. 3, the caval venous pressure

TABLE 1. *Effect of morphine and ten related substances on the blood pressure and on four smooth muscle preparations in the cat and tachyphylaxis (t) to these substances compared with their effects on the responses to adrenaline (A), noradrenaline (NA) and angiotensin (Ang.)*

Drug	§ Dose range (mg/kg)	Blood pressure					Nictitating membrane					Uterus (non-gravid)					Urinary bladder					Small intestine				
		Effect	t	A	NA	Ang.	Effect	t	A	NA	Ang.	Effect	t	A	NA	Ang.	Effect	t	A	NA	Ang.	Effect	t	A	NA	Ang.
Morphine	3–36	D	+	D	D	D	I	+	DP	DP		I	+	D	D				D	D	D	V		D	D	D
Nalorphine	10	D			D	D														0	0				D	D
Normorphine	5–40	D		D	D	D								0	0				0	0				0	0	
Ethylmorphine	2–4	†D	+	D	D	D							0	0	0	0	V	+‡	D	D	D					
3-hydroxymorphinan	10	†D/I‡	+‡	DP	DP	D						I	0	0	0	0	I	0	I	I	I	I	+	I	I	0
Methadone	5–10	†D	0	DP	DP	D	I		DP	DP		I	0	IP‡	IP‡	V	I	+	P	P	0	I	+	P	P	0
Pethidine	5–10	D	0‡	DP	DP							0		0	0		I	+	D	P		D	0	0	0	
Levallorphan	2·5–7·5	†D	0	DP	DP	D	I		DP	D			0	P	0	D		0	P	P	0		0	P	P	P
N-allylbenzomorphan*	2·5–5·0	D	0	DP	DP	D						I	0	P	0	0	I	0	P	P	0	I	0	P	0	0
Etonitazene**	4–5	D	0	DP	DP	D						0		P	0	0	0		0	0	0	I	+	0	0	0
Dextromoramide	2·5–5·0	D	0	DP	DP	D						I	0	0	0	0	D	0	0	0	0					

*SKF 10047. ** 1-(β-diethylaminoethyl)-2-(p-ethoxybenzyl)-5-benzimidazole.

Effects: D, decreased (blood pressure: contraction or tone of smooth muscle preparation); I, increased; P, prolonged; V, variable; 0, no effect. t, + = tachyphylaxis; t, 0 = absence of tachyphylaxis.

§ Usually repeated, see **Methods** and legend to Fig. 3.

† Repeated doses produced cardiac arrhythmias.

‡ See **Results** (*Effects of other related substances*).

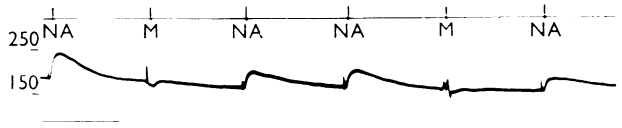

FIG. 1. Depressant effect of morphine on the vasoconstrictor action of noradrenaline. Cat, 3·4 kg. Tracing: Changes in the hindquarter perfusion pressure (in mmHg) at a constant perfusion rate of 36 ml/min. Upper line: noradrenaline (NA), 20 μg; morphine (M), first injection 50 mg, second injection 100 mg. Lower line: time calibration, 4 min.

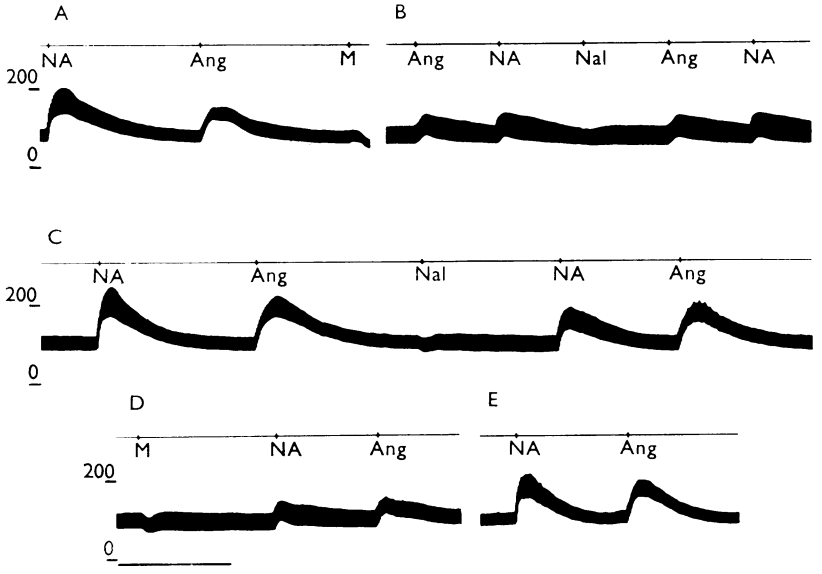

FIG. 2. Reduction by morphine and/or nalorphine of the pressor responses to noradrenaline and angiotensin. Blood pressure in mmHg. A–B: cat, 3 kg. Drugs: noradrenaline (NA), 20 μg; angiotensin (Ang), 2 μg; morphine (M), 10 mg/kg; nalorphine (Nal), 10 mg/kg. Between A and B, 7 min. C–E: cat, 2·7 kg. Noradrenaline (NA), 15 μg; angiotensin (Ang), 1·5 μg; nalorphine (Nal), 10 mg/kg; morphine (M), 10 mg/kg. Between C and D, 4·5 min; between D and E, 28 min, during which two injections of noradrenaline were made. Time calibration, 4 min.

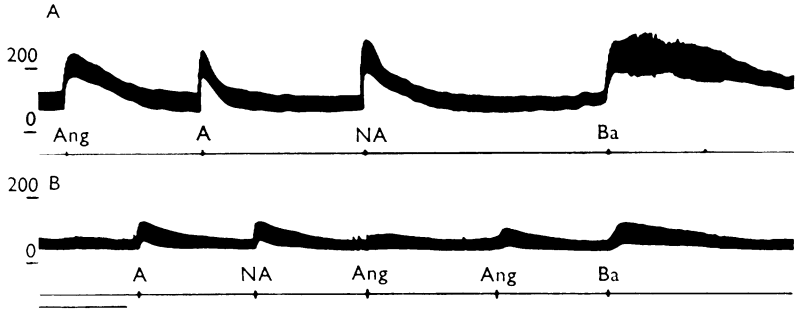

FIG. 3. Cat, 2·3 kg. Reduction by morphine (scheme below) of the pressor responses (in mmHg) to angiotensin (Ang), 1 μg (the third injection was 2 μg); adrenaline (A), 15 μg; noradrenaline (NA), 20 μg; barium chloride (Ba), 4·6 mg. Between A and B, 69 min, morphine was administered at 4–5 min intervals as follows: two injections of 3 mg/kg; three injections of 6 mg/kg; four injections of 12 mg/kg; three injections of 24 mg/kg and three injections of 36 mg/kg. Time calibration, 3 min.

and left ventricular end-diastolic pressure remained unchanged throughout the course of the treatment. Further, noradrenaline or angiotensin injected at any time during this period did not produce an increase in either pressure. In the perfused hindquarters (Fig. 1), successive doses of morphine diminished the increase in vascular resistance produced by a constant dose of noradrenaline. Morphine caused hypotension (Fig. 2) and this effect showed tachyphylaxis (Table 1). In addition, the alkaloid reduced the pressor responses to adrenaline, noradrenaline, angiotensin and barium chloride (Figs. 2–5 and Table 1). Nalorphine did not antagonize the depressor effect of a similar dose of morphine but itself had a slight hypotensive action and also, like morphine, reduced the pressor responses to noradrenaline and angiotensin.

Tibialis anterior responses. Morphine greatly reduced the responses of the denervated tibialis anterior muscle to acetylcholine, carbachol and adrenaline (Fig. 4). The effects of direct or indirect stimulation of the muscle were also markedly decreased but the potentiating action of adrenaline on the directly elicited twitches was still prominent after the morphine treatment (Fig. 5).

Smooth muscle responses. Figure 6 shows that on the nictitating membrane and non-gravid uterus, morphine produced a contraction to which tachyphylaxis occurred. If given before adrenaline or noradrenaline, morphine reduced but prolonged their effects on the nictitating membrane but only diminished them on the uterus. The activity of the urinary bladder was only marginally affected by morphine which did, however, depress the responses to adrenaline, noradrenaline and angiotensin, subsequently administered (Table 1). The spontaneous activity of the small intestine varied considerably during the morphine treatment which greatly diminished the responses to subsequent injections of adrenaline, noradrenaline, angiotensin and barium chloride (Fig. 7). None of these effects of morphine were prevented by nalorphine (5–10 mg/kg). This drug, like morphine, usually depressed

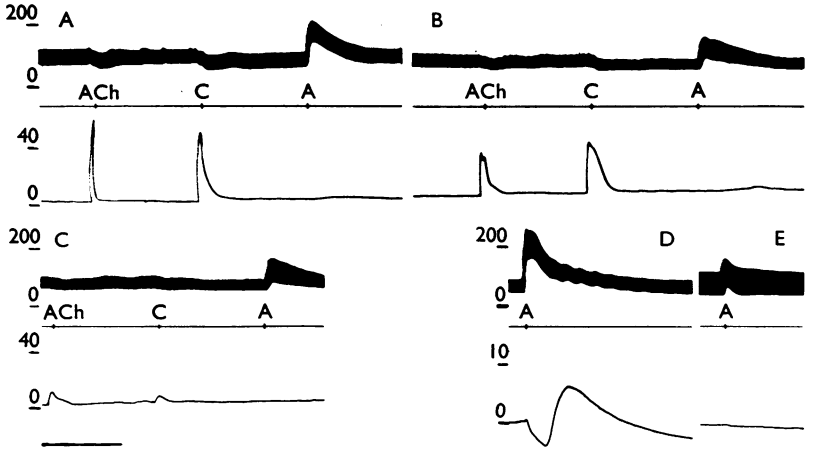

FIG. 4. Depressant action of morphine on the cardiovascular and skeletal muscle effects produced by acetylcholine, carbachol and adrenaline. Upper thick line, blood pressure in mmHg; lower thin line, contraction of tibialis anterior (tension in g) denervated 8 (first cat) or 9 days earlier. A–C: cat, 3·1 kg. Acetylcholine (ACh), 2 μg; carbachol (C), 6·25 μg; adrenaline (A), 15 μg. Between A and B, 22 min: morphine, three injections of 10 mg/kg and three injections of 20 mg/kg; between B and C, 12 min: morphine, one injection of 20 mg/kg and two injections of 30 mg/kg. D–E: cat, 2·3 kg. Adrenaline (A) 15 μg. Between D and E, 45 min: morphine, two injections of 10 mg/kg. Time calibration, 3 min.

agonist activity although it was ineffective against noradrenaline and angiotensin on the bladder (Table 1).

Effects of other related substances

Cardiovascular actions. Pethidine (5–10 mg/kg) produced a marked hypotension, with a decrease in pulse pressure, which lasted for 2–3 min: the fall in blood

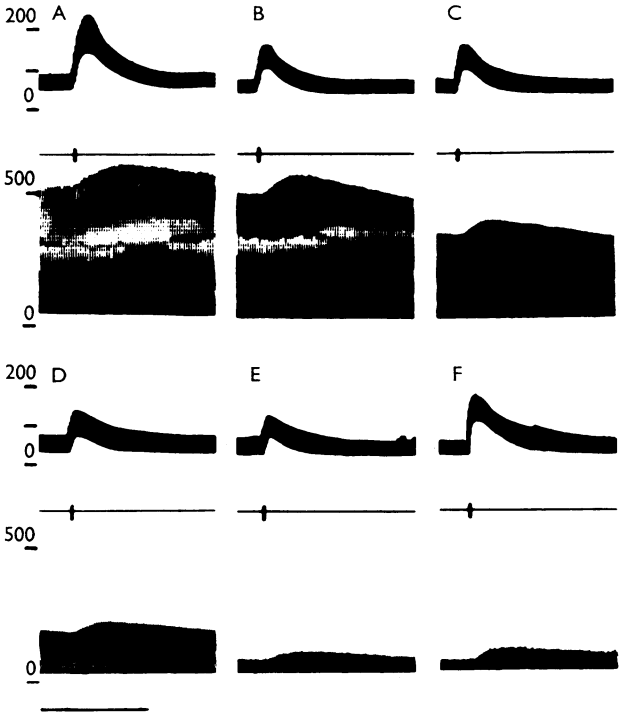

FIG. 5. Effects of morphine on the responses of the tibialis anterior preparation to direct stimulation and to adrenaline administration. Cat, 2·6 kg: tibialis anterior denervated 7 days earlier. Upper line, blood pressure in mmHg; middle line, at signals, adrenaline, 15 μg (except F which was 60 μg); lower line, muscular twitches (in g tension). Between A and B, 15 min: morphine, one injection of 10 mg/kg, one injection of 15 mg/kg and one injection of 20 mg/kg. Between B and C, 8 min: morphine, one injection of 20 mg/kg and one injection of 30 mg/kg. Between C and D, 12 min: morphine, three injections of 30 mg/kg. Between D and E, 23 min, and between E and F, 6 min, no injections. Time calibration, 3 min.

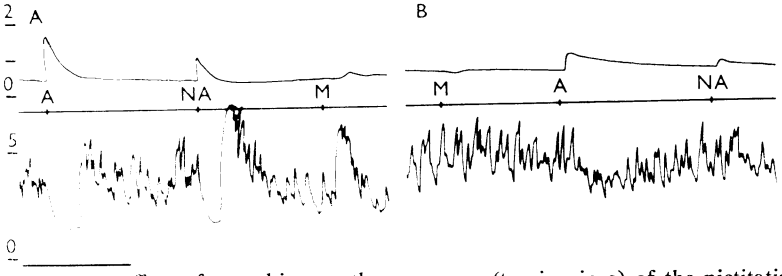

FIG. 6. Depressant effect of morphine on the responses (tension in g) of the nictitating membrane (upper tracing) and non-gravid uterus (lower tracing) to adrenaline and noradrenaline. Cat, 1·8 kg. Middle line: adrenaline (A), 15 μg; noradrenaline (NA), 20 μg; morphine (M), first injection 3 mg/kg, last injection 36 mg/kg, between A and B, 66 min, one injection of 3 mg/kg, four injections of 6 mg/kg, three injections of 12 mg/kg, four injections of 24 mg/kg, two injections of 36 mg/kg. Time calibration, 4 min.

pressure became *greater* on repeated injection of the drug. With 3-hydroxymorphinan (10 mg/kg) a small, evanescent hypotension was succeeded by a more prolonged pressor phase (40–60 mmHg): on repetition of the dose, the depressor effect became greater but the rise in blood pressure showed tachyphylaxis.

Smooth muscle responses. On the uterus, methadone increased and prolonged the relaxant effects of adrenaline, noradrenaline and, possibly, angiotensin (Fig. 8). Ethylmorphine had a variable effect on the cat bladder, either decreasing the tone or producing a contraction; only the latter showed tachyphylaxis on repeated administration of the drug.

Discussion

We have again confirmed the tachyphylaxis produced by repeated doses of morphine on the cat blood pressure and the concomitant decrease in the pressor response to noradrenaline during the period of morphine hyposensitivity. Our suggestion (Huidobro *et al.*, 1968) that the diminution in the responses might be due to progressive myocardial depression has not been substantiated. Firstly because, during repeated dosage with morphine, the characteristic signs of heart failure, that is a concurrent venous hypertension and increased left ventricular end-diastolic pressure, were not observed. Secondly, the decreased response to noradrenaline still occurred in the perfused hindquarter preparation in the presence of a constant blood flow at an adequate perfusion pressure. The latter observation also excludes a prolonged depressant action of morphine on the peripheral circulation of the hindquarters as a cause of the noradrenaline hyposensitivity.

By widening the scope of the investigation to include the effects of repeated doses of morphine on a number of preparations in the spinal cat (blood pressure, tibialis anterior, nictitating membrane, uterus, bladder and small intestine) a parallelism between morphine tachyphylaxis and decreased responses to adrenaline, noradrenaline, angiotensin and, possibly, barium chloride, acetylcholine and carbachol is apparent (see Table 2). The range of substances to which hyposensitivity was manifest (morphine, catecholamines, barium ions and choline esters) seems to indicate that the action is either non-specific at receptor level or affecting

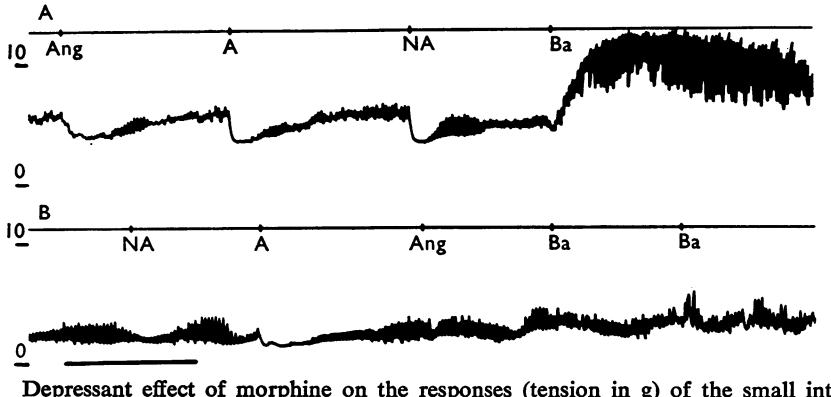

FIG. 7. Depressant effect of morphine on the responses (tension in g) of the small intestine. Cat, 2·3 kg. Drugs: upper line, angiotensin (Ang) 1·5 μg; adrenaline (A) 15 μg; noradrenaline (NA) 20 μg; barium chloride (Ba) 4·6 mg: between A and B, 88 min, morphine, two injections of 3 mg/kg, four injections of 12 mg/kg, four injections of 24 mg/kg and two injections of 36 mg/kg: lower line, angiotensin 3 μg; adrenaline 45 μg, noradrenaline 20 μg; barium chloride 4·6 and 9·2 μg. Time calibration, 4 min.

post-receptor processes. The fact that the decreased responses to these drugs occurred in all the preparations tested (which reacted in different ways to the substances related to morphine, Table 2) might indicate some basic underlying process which could possibly be related to morphine hyposensitivity within the central nervous system. Just as tolerance is not shown clinically to all the central actions of morphine, in the present paper there is one action which is not suppressed, namely the potentiating action of adrenaline on the tibialis anterior response to direct muscle stimulation (Huidobro, Cubillos & Eyzaguirre, 1952). Morphine has already been shown to have a number of depressant actions: for example, on cerebral metabolism (Takemori, 1962 ; Clouet, Ratner & Williams, 1966 ; Clouet & Ratner, 1968): on the responses of the nictitating membrane to pre- or post-ganglionic stimulation (Trendelenburg, 1957 ; Cairnie, Kosterlitz & Taylor, 1961 ; Gyang, Kosterlitz & Lees, 1964) ; on the cardiac responses to vagal stimulation (Kosterlitz & Taylor, 1959 ; Kennedy & West, 1967) and on the release of the chemical transmitter, acetylcholine (Paton, 1957 ; Schaumann, 1957). Morphine can suppress the actions of a number of drugs at the peripheral receptors (Trendelenburg, 1957 ; Kosterlitz & Robinson, 1958). However, Trendelenburg

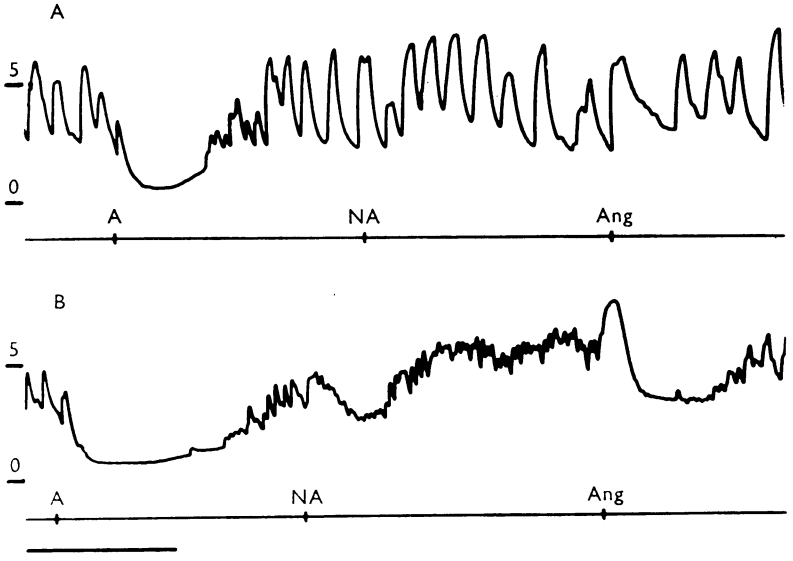

FIG. 8. Potentiation by methadone of the responses (tension in g) of the uterus to adrenaline, noradrenaline and angiotensin. Cat, 2·7 kg. Angiotensin (Ang), 2·5 μg ; adrenaline (A), 15 μg ; noradrenaline (NA), 15 μg. Between A and B, 21 min: methadone, one injection of 10 mg/kg and two injections of 5 mg/kg. Time calibration, 3 min.

TABLE 2. *Number of times tachyphylaxis (t) to either morphine or one of ten related substances was present (+) or absent (o) concomitantly with one of the following responses to adrenaline, noradrenaline or angiotensin in the same cat preparation: decreased* (D)*; prolonged* (P)*; no effect (O); variable* (V)*; increased* (I).

		Effect on responses to adrenaline, noradrenaline or angiotensin						
Drug	t	D	DP	P	O	V	I	IP
Morphine	+	5	2					
	o							
One of the ten related substances	+	8	2	5	6		2	
	o	6	12	10	21	1	3	2

(1957) and Cairnie *et al.* (1961) found that morphine did not decrease the nictitating membrane contraction induced by adrenaline or noradrenaline. This fact does not agree with our results but we used repeated doses larger than the single ones employed by these earlier workers.

All the substances related to morphine which were tested decreased the pressor actions of adrenaline, noradrenaline and angiotensin and some, in addition, prolonged the responses to the catecholamines but these effects did not bear any relationship to the production of tachyphylaxis (Table 1). When the experiments were repeated in the smooth muscle preparations, it was obvious that there was no parallelism between the production of hyposensitivity to these substances related to morphine and a decreased response to either adrenaline, noradrenaline or angiotensin (Table 2). Tachyphylaxis could occur without, or be absent concomitantly with, decreased sensitivity to the catecholamines and angiotensin. Further, prolongation of the responses to these latter substances is specific neither to the desensitizing substance nor to the preparation under test. Perhaps these substances, and possibly also morphine, act on different morphological or functional receptors in the various tissues of the cat.

The authors wish to express their gratitude to Professor C. Eyzaguirre for his invaluable suggestions and criticism and to Dr. N. F. Grundy for his assistance in the preparation of the paper for publication. This work was partially aided by a grant from the Gildmeister Foundation, Santiago, Chile.

REFERENCES

CAIRNIE, A. B., KOSTERLITZ, H. W. & TAYLOR, D. W. (1961). Effect of morphine on some sympathetically innervated effectors. *Br. J. Pharmac. Chemother.*, **17**, 539–551.

CLOUET, D. H., RATNER, M. & WILLIAMS, N. (1966). C^{14} leucine and C^{14}phenylalanine incorporation into brain ribosomes. *Fedn Proc.*, **25**, 773.

CLOUET, D. H. & RATNER, M. (1968). The effect of morphine administration on the incorporation of [^{14}C] leucine into protein in cell-free systems from rat brain and liver. *J. Neurochem.*, **15**, 17–23.

EVANS, A. G. J., NASMYTH, P. A. & STEWART, H. C. (1952). The fall of blood pressure caused by intravenous morphine in the rat and the cat. *Br. J. Pharmac. Chemother.*, **7**, 542–552.

GYANG, E. A., KOSTERLITZ, H. W. & LEES, G. M. (1964). The inhibition of autonomic neuroeffector transmission by morphine-like drugs and its use as a screening test for narcotic analgesic drugs. *Arch. exp. Path. Pharmak.*, **248**, 231–246.

HUIDOBRO, F., CUBILLOS, L. & EYZAGUIRRE, C. (1952). Effects of aromatic amines on neuromuscular function. *Acta Physiol. latinoamer.*, **2**, 107–118.

HUIDOBRO, F., HUIDOBRO, J. P. & LEWIN, J. (1968). Some features about tachyphylaxis. *Archs int. Pharmacodyn. Thér.*, **175**, 391–400.

KENNEDY, B. L. & WEST, T. C. (1967). Effect of morphine on electrically-induced release of autonomic mediators in the rabbit sinoatrial node. *J. Pharmac. exp. Ther.*, **157**, 149–158.

KOSTERLITZ, H. W. & ROBINSON, J. A. (1958). The inhibitory action of morphine on the contraction of the longitudinal muscle coat of the isolated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **13**, 296–303.

KOSTERLITZ, H. W. & TAYLOR, D. W. (1959). The effect of morphine on vagal inhibition of the heart. *Br. J. Pharmac. Chemother.*, **14**, 209–214.

PATON, W. D. M. (1957). The action of morphine and related substances on the contraction and on the acetylcholine output of coaxially stimulated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **12**, 119–127.

SCHAUMANN, W. (1957). Inhibition by morphine of the release of acetylcholine from the intestine of the guinea-pig. *Br. J. Pharmac. Chemother.*, **12**, 115–118.

TAKEMORI, A. E. (1962). Studies on cellular adaptation to morphine and its reversal by nalorphine in cerebral cortical slices of rats. *J. Pharmac. exp. Ther.*, **135**, 89–93.

TRENDELENBURG, U. (1957). The action of morphine on the superior cervical ganglion and on the nictitating membrane of the cat. *Br. J. Pharmac. Chemother.*, **12**, 79–85.

(*Received January* 8, 1970)