Br. J. Pharmac. (1976), **57**, 551–557

THE EFFECTS OF MORPHINE ON THE RELEASE OF NORADRENALINE FROM THE MOUSE VAS DEFERENS

G. HENDERSON & J. HUGHES
Unit for Research on Addictive Drugs, Marischal College, University of Aberdeen

1 Electrical field stimulation of the mouse vas deferens (TO and C57/BL strains) caused the release of noradrenaline into the bathing medium.

2 Phenoxybenzamine (30 μM) or phentolamine (36 μM) plus cocaine (13 μM) caused a considerable increase in the noradrenaline output.

3 In the vasa deferentia from TO mice the output per pulse of noradrenaline was constant at frequencies of stimulation from 0.5 to 15 Hz whereas in the vasa deferentia from C57/BL mice the output per pulse of noradrenaline increased two-fold from 1.5 to 15 Hz.

4 Morphine (2 μM) inhibited the contractions of the vasa deferentia from TO mice. This effect was greater at low (0.1–1 Hz) than at high (10 Hz) frequencies of stimulation. Morphine (2 μM) did not inhibit the response of the tissue to exogenous noradrenaline.

5 Morphine (1 μM) reduced the noradrenaline output from the vasa deferentia of TO mice stimulated at 1.5 Hz but did not reduce the noradrenaline output at 15 Hz. At 1.5 Hz the reduction of noradrenaline output was reversed by naloxone (0.05 μM).

6 Morphine (5 μM) did not inhibit the uptake of [^{3}H]-noradrenaline into the vasa deferentia from TO mice.

7 Only in high concentrations (ID_{50} 30.88 μM) did morphine inhibit the contractions of the vasa deferentia from C57/BL mice.

8 Normorphine (100 μM) did not reduce the noradrenaline output from vasa deferentia of C57/BL mice.

Introduction

Morphine and its cogeners have been shown to inhibit noradrenaline release in the cat nictitating membrane (Henderson, Hughes & Kosterlitz, 1975) and in the mouse vas deferens (Henderson, Hughes & Kosterlitz, 1972; Hughes, Kosterlitz & Leslie, 1975). In the cat nictitating membrane the output of noradrenaline is constant over the frequency range 0.2–15 Hz whereas at morphine-insensitive adrenergic junctions the output of noradrenaline increases as the frequency of nerve stimulation is increased (Hughes, 1972; Hughes & Roth, 1974; Henderson *et al.*, 1975). Similarly, the relationship between acetylcholine output and frequency of stimulation is different at morphine-sensitive and morphine-insensitive peripheral cholinergic junctions (Greenberg, Kosterlitz & Waterfield, 1970). Inhibition of noradrenaline release by narcotic analgesics has been demonstrated in rat cerebral cortex slices (Montel, Starke & Weber, 1974); in this preparation the noradrenaline output was greater at low (0.3 Hz) than at high (10 Hz) frequencies of stimulation. The purpose of this investigation was to determine whether the noradrenaline output from the mouse vas deferens shows a similar relationship with respect to stimulation frequency as that previously observed at other morphine-sensitive junctions.

A preliminary report of these results has been made to the British Pharmacological Society (Henderson & Hughes, 1975).

Methods

Mouse vas deferens

Two strains of mice were used; TO and C57/BL. Male mice weighing 25–35 g were killed by cervical dislocation; the vasa deferentia connected by a small portion of seminal vesicle were dissected out and placed in Krebs solution. The semen was gently

expressed from the lumen and the tissue mounted vertically, under 0.5–1 g tension, in a 2.5 ml organ bath containing Krebs solution at 37°C. In those experiments in which the noradrenaline output from the vas deferens was measured, 4–6 pairs of vasa deferentia were mounted in a single organ bath.

Nerve stimulation

Electrical field stimulation was used to excite the intramural nerves. The cathode consisted of a ring of platinum foil (5 mm in depth) fixed to the top of the bath; the anode consisted of a fine coil of platinum wire inserted through the base of the bath. Supramaximal stimuli (1.3 × current required to produce the maximum mechanical response of the tissue) of 0.5–2 ms duration rectilinear pulses were used throughout. The contractions of the tissue to nerve stimulation were recorded isometrically and displayed on a pen oscillograph.

Determination of noradrenaline

The methods used to determine the tissue content of noradrenaline and the amount of noradrenaline released from the vas deferens by nerve stimulation were as described by Henderson *et al.* (1975). In several experiments the noradrenaline released from the vas deferens was estimated fluorimetrically by the method of O'Hanlon, Campuzano & Horvath (1970) without first being concentrated by alumina chromatography. No difference was observed between results obtained with or without alumina chromatography.

The vasa deferentia were stimulated at 15–30 min intervals with trains of 240 pulses. After nerve stimulation the fluid surrounding the vasa was left for 3 min (non-pretreated preparations) or 5 min (tissues pretreated with phenoxybenzamine or phentolamine plus cocaine) to permit the released noradrenaline to diffuse from the tissue into the surrounding bath fluid. The values for noradrenaline output after nerve stimulation have been corrected for the spontaneous output of noradrenaline during the collection period. Experiments in which the stimulated output of noradrenaline from the tissues declined by more than 10% per stimulus train were rejected. In any one experiment the output of noradrenaline at a single frequency is the mean of at least two observations. As described previously (Henderson *et al.*, 1975) electrical field stimulation did not produce any destruction of noradrenaline added to the organ bath.

Determination of [^{3}H]-noradrenaline uptake

Vasa deferentia were incubated at 37°C in Krebs solution containing 0.059 μM [^{3}H]-noradrenaline (specific activity 6.76 Ci/mmol) for various periods of time. After incubation, surface moisture was removed, the tissues homogenized and the noradrenaline extracted and purified as described by Henderson *et al.* (1975). The purified noradrenaline was added to 20 ml scintillation fluid. All samples were counted in a Packard Liquid Scintillation Counter for 10 min, with a counting efficiency 20–25%.

Drugs and solutions

The bathing fluid was a modified Krebs solution of the following composition (mM): NaCl 118, KCl 4.75, $CaCl_2$ 2.54, $MgSO_4$ 1.19, $NaHCO_3$ 24, KH_2PO_4 0.93, glucose 11, tyrosine 0.25, ascorbic acid 0.1 and disodium edetate 0.027; it was bubbled with 95% O_2 plus 5% CO_2.

Table 1 Fractional noradrenaline output per pulse from the mouse vas deferens due to stimulation at 0.5, 1.5 or 15 Hz with trains of 240 pulses

Treatment	*Fractional noradrenaline release per pulse (×10^5) at*			
	0.5 Hz	*1.5 Hz*	*15 Hz*	
(a) TO mice				
(i) None	0.83 ± 0.16	0.66 ± 0.13	0.71 ± 0.15	
(ii) Phenoxybenzamine, 30 μM	6.47 ± 0.62	7.73 ± 0.97	9.16 ± 0.94	
(iii) Phentolamine, 36 μM plus cocaine, 13 μM		4.83 ± 0.85	5.62 ± 0.91	
(b) C57/BL mice				
Phentolamine, 36 μM plus cocaine, 13 μM		1.78 ± 0.26	3.65 ± 0.76	($P < 0.0125$)

The tissues were incubated with drug solutions for 1 h before stimulation. The noradrenaline outputs from untreated and phenoxybenzamine-treated tissues were measured by biological assay; the noradrenaline outputs from phentolamine and cocaine-treated tissues were measured by fluorimetric assay. The values are the means ± s.e. of 4 experiments.

The drugs used were cocaine hydrochloride (Paterson), morphine hydrochloride (Macfarlan Smith), naloxone hydrochloride (Endo Laboratories), (—)-noradrenaline bitartrate (BDH), normorphine hydrochloride (Dr E. L. May), phenoxybenzamine hydrochloride (Smith, Kline & French) and phentolamine hydrochloride (Ciba). A conventional dioxane-toluene scintillation fluid was used.

Results

Noradrenaline output

(a) TO mice. The noradrenaline content of the vas deferens was 4.95 ± 0.39 μg/g tissue ($n = 11$). After stimulation with trains of 240 pulses, the fractional noradrenaline output per pulse from the untreated vasa deferentia was not significantly different at 0.5, 1.5 and 15 Hz (Table 1). The noradrenaline output from tissues incubated with phenoxybenzamine (30 μM) for 1 h was between 8 and 13-fold greater than that from untreated preparations (Table 1). After phenoxybenzamine the noradrenaline output was slightly higher at 15 Hz than at 0.5 Hz. In tissues which had been incubated with phentolamine (36 μM) plus cocaine (13 μM) for 1 h there was no significant increase in the noradrenaline output per pulse between 1.5 and 15 Hz (Table 1).

(b) C57/BL mice. The noradrenaline content of the vas deferens was 4.36 ± 0.16 μg/g tissue ($n = 8$). In the vasa deferentia of this strain of mice the noradrenaline frequency output relationship after stimulation with trains of 240 pulses at 1.5 and 15 Hz was different from that observed in the vas deferens from TO mice. After exposure of the vas deferens from C57/BL mice to phentolamine (36 μM) plus cocaine (13 μM) for 1 h the fractional noradrenaline output per pulse at 15 Hz was twice that at 1.5 Hz (Table 1).

Effect of morphine on contractions of the vas deferens

(a) TO mice. The contractions of the vas deferens from this strain of mice due to stimulation at 0.1 Hz were inhibited by morphine in a dose-dependent manner (Figure 1). As previously described, the minimum concentration of morphine required to inhibit the contraction at 0.1 Hz was 0.05 μM; 50% of the total contraction was inhibited by 0.47 ± 0.03 μM; concentrations of morphine greater than 5 μM produced a maximum inhibition of the contraction (Henderson *et al.*, 1972). In all preparations tested, normorphine was equiactive with morphine in depressing the contraction. The inhibition of contraction produced by morphine was completely reversible by naloxone. Naloxone (0.3–0.9 μM) did not itself alter the contractions of the vas deferens.

A comparison of the effects of a single concentration of morphine (2 μM) on the responses due to stimulation at various frequencies showed that morphine produces a greater inhibition of the contractions at low (0.1–1 Hz) than at high (10 Hz) frequencies of stimulation (Figure 2a, $n = 3$). Morphine (2 μM) did not alter the response of the vas deferens to exogenous noradrenaline whereas the response to

Table 2 Fractional noradrenaline output per pulse from the mouse vas deferens due to stimulation at 1.5 and 15 Hz with trains of 240 pulses before and after exposure to morphine and naloxone

Treatment	*Fractional noradrenaline release per pulse ($\times 10^5$) at 1.5 Hz*		*15 Hz*
(a) TO mice			
(i) None	1.72 ± 0.27 (5)	$P < 0.01$	1.66 ± 0.21 (4)
Morphine, 1 μM	0.77 ± 0.15 (5)		1.37 ± 0.12 (4)
Morphine, 1 μM plus naloxone, 0.05 μM	1.49 ± 0.15 (5)	$P < 0.025$	1.46 ± 0.20 (4)
(ii) None	1.84 ± 0.22 (4)		1.80 ± 0.31 (4)
Naloxone, 0.05 μM	1.83 ± 0.33 (4)		1.78 ± 0.12 (4)
(b) C57/BL mice			
None	4.95 ± 0.76 (4)		
Normorphine, 100 μM	3.24 ± 0.70 (4)		

All tissues were pretreated with phentolamine 36 μM plus cocaine 13 μM for 1 hour. The tissues were exposed to morphine for 6 min and to naloxone for 20 min before stimulation. The noradrenaline outputs were assayed fluorimetrically. The results were obtained from paired observations. The values are the means ± s.e.; the numbers in parentheses are the number of observations. Fractional noradrenaline output per pulse is the output as a fraction of the total tissue content of noradrenaline after completion of the experiment.

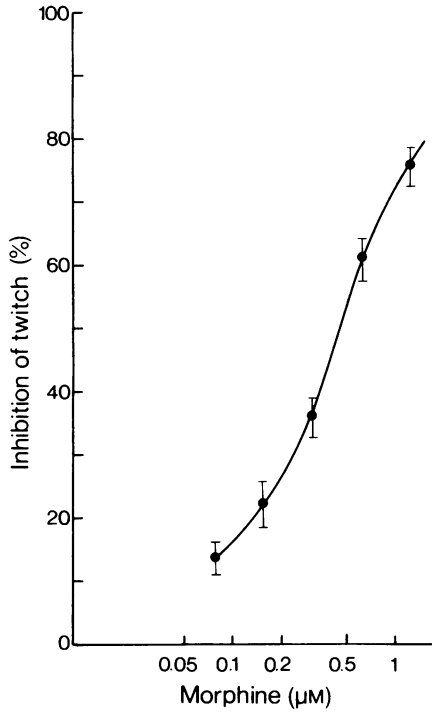

Figure 1 Effect of morphine on contractions of the mouse (TO) vas deferens stimulated continuously at 0.1 Hz. Mean results from 6 experiments. Vertical lines show s.e. mean. Ordinate scale: inhibition of twitch as a percentage of the initial response. Abscissa scale: morphine concentration.

electrical stimulation (0.1 Hz) was depressed by 75–85% (Figure 2b, $n=3$).

(b) C57/BL mice. The contractions due to stimulation at 0.1 Hz of the vas deferens from this strain of mice were not inhibited by morphine or normorphine until concentrations of 3–10 μM were used. Attempts were made to construct dose-response curves for morphine against contractions at 0.1 Hz but in only 4 out of 10 experiments was the maximum inhibition obtained greater than 50% of the initial contraction. In these 4 experiments the concentration of morphine required to produce 50% inhibition of the initial contraction ranged from 12–47 μM with a mean of 30.88 ± 9.47 μM. Naloxone (2.5 μM) completely reversed the inhibition of contraction produced by normorphine (50 μM, $n=4$, Figure 3). This concentration of naloxone did not alter the response of the vas deferens to exogenous noradrenaline.

Effect of morphine on the noradrenaline output from the vas deferens

Since there was considerable variation in the noradrenaline outputs of different preparations, these results were obtained from paired observations.

(a) TO mice. Exposure of the vas deferens to morphine (1 μM) for 6 min before stimulation resulted in a 55% depression of the fractional noradrenaline output per pulse at 1.5 Hz (Table 2). The fractional noradrenaline output per pulse at 15 Hz was not

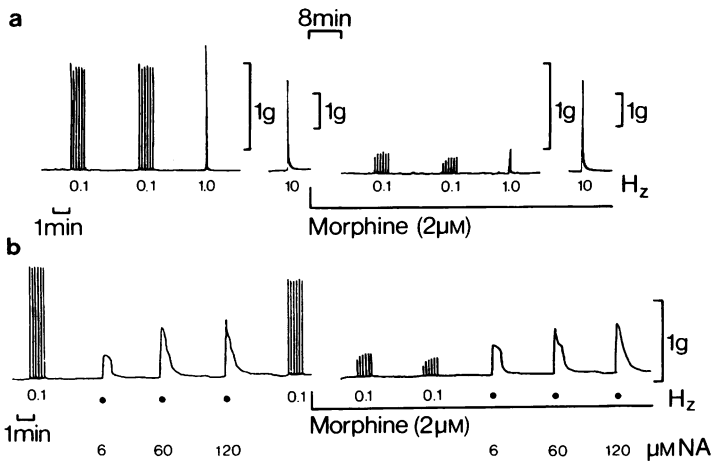

Figure 2 Effect of morphine on the responses of the mouse (TO) vas deferens to nerve stimulation or to exogenous noradrenaline. (a) The effect of morphine on the response to nerve stimulation was greater at low (0.1–1 Hz) than at high (10 Hz) frequencies; (b) Morphine did not alter the response to exogenous noradrenaline (NA) whereas the response to nerve stimulation was inhibited.

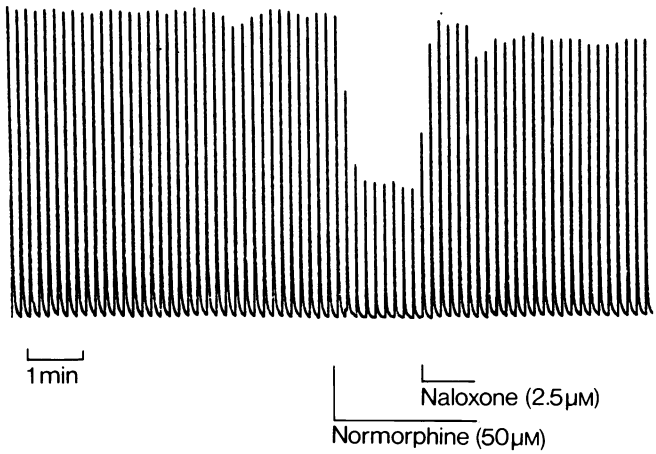

Figure 3 Effect of normorphine on the responses of the mouse (C57/BL) vas deferens to nerve stimulation at 0.1 Hz. Naloxone reversed the inhibition produced by normorphine.

significantly altered (Table 2). The depression by morphine of the noradrenaline output at 1.5 Hz was prevented by pretreatment of the tissue with naloxone (0.05 μM) for 20 min whereas naloxone (0.05 μM) itself did not alter the noradrenaline output from the vas deferens (Table 2).

(b) C57/BL mice. Exposure of the vas deferens to normorphine (100 μM) for 6 min prior to stimulation did not significantly alter the noradrenaline output at 1.5 Hz (Table 2).

Effect of morphine on [^{3}H]-noradrenaline uptake

Vasa deferentia from TO mice were incubated in Krebs solution containing [^{3}H]-noradrenaline (0.059 μM) for 0, 2 and 5 min; the [^{3}H]-noradrenaline was actively taken up and concentrated in the tissue. There was no difference at 2 and 5 min between the [^{3}H]-noradrenaline uptake into tissues incubated with [^{3}H]-noradrenaline alone and tissues incubated with [^{3}H]-noradrenaline plus morphine (5 μM) (Table 3).

Discussion

We believe that the motor innervation of the mouse vas deferens is adrenergic and that morphine acts by inhibiting the release of noradrenaline from the motor nerve terminals. The adrenergic nature of the innervation has been questioned (Ambache & Zar, 1971; Jenkins, Marshall & Nasmyth, 1975) and it is true that the vasa deferentia of several species exhibit anomalous pharmacological responses, particularly in respect of α-adrenoceptor blocking agents. We attribute these anomalous responses to the dense nature of the adrenergic innervation and in particular to the very close apposition of nerve endings and smooth muscle cells in this type of tissue (Lane & Rhodin, 1964; Yamauchi & Burnstock, 1969; Furness & Iwayama, 1971). The evidence in favour of adrenergic motor transmission is considerable; thus bretylium (Hughes, 1972), lysergic acid diethylamide (Hughes, 1973), and morphine (Henderson *et al.*, 1972; Hughes *et al.*, 1975) all inhibit noradrenaline release in the vas deferens by different mechanisms and also inhibit contractions of the tissue to nerve

Table 3 Effect of morphine on [^{3}H]-noradrenaline uptake into the mouse (TO) vas deferens

Treatment	*[^{3}H]-noradrenaline uptake (d min^{-1} g^{-1} tissue × 10^4) at*	
	2 min	*5 min*
None	68.6 ± 2.1	99.9 ± 3.6
Morphine, 5 μM	63.9 ± 4.7	102.3 ± 9.0

Tissues were incubated with [^{3}H]-noradrenaline, 0.059 μM (specific activity 6.76 Ci/mmol). The values are the means ± s.e. of 3 observations.

stimulation. Also, it has been shown that in the mouse vas deferens both guanethidine and 6-hydroxydopamine block motor responses to nerve stimulation (Furness, Campbell, Gillard, Malmfors, Cobb & Burnstock, 1970; Jones & Spriggs, 1975).

There is no evidence for the presence of ganglion synapses within the smooth muscle of the vas deferens or for a cholinergic component in the release of noradrenaline. The contractions and noradrenaline output of the vas deferens are unaffected by exposure of the tissue to hexamethonium (490 μM), hyoscine (0.23 μM) or eserine (7.7 μM) (Henderson *et al.*, 1972; Henderson, 1974). Thus, since morphine inhibits the response to nerve stimulation but not the response to exogenous noradrenaline the site of action of morphine must be on the postganglionic sympathetic nerve fibre to inhibit directly the release of noradrenaline as in the cat nictitating membrane (Henderson *et al.*, 1975). Intracellular recording from the smooth muscle of the mouse vas deferens during nerve stimulation has demonstrated the presynaptic site of action of morphine (North & Henderson, 1975; Henderson & North, 1976).

Using similar experimental procedures the characteristics of noradrenaline release have been examined from two tissues in which the noradrenaline output is inhibited by morphine, i.e. the mouse (TO) vas deferens (this paper) and cat nictitating membrane (Henderson *et al.*, 1975), and from four tissues in which the noradrenaline output is not inhibited by morphine, i.e. mouse (C57/BL) vas deferens (this paper), guinea-pig ileum myenteric plexus-longitudinal muscle preparation (Henderson *et al.*, 1975), rabbit portal vein and vas deferens (Hughes, 1972; Hughes & Roth, 1974). Within each group the characteristics of noradrenaline release are very similar. In the morphine-sensitive tissues the fractional noradrenaline output per pulse is constant over a wide range of frequencies (0.2–15 Hz for the nictitating membrane and 0.5–15 Hz for the vas deferens). In these tissues, inhibition of neuronal and extraneuronal noradrenaline uptake and inhibition of pre- and post-junctional α-adrenoceptors by phenoxybenzamine or phentolamine plus cocaine increased the noradrenaline output at each frequency of stimulation but did not greatly alter the relationship between noradrenaline output and frequency of stimulation. Therefore this relationship appears to reflect directly the properties of the noradrenaline release process. In the mouse (C57/BL) vas deferens, as in the other morphine-insensitive tissues, the noradrenaline output is not constant over a range of frequencies but increases as the frequency of stimulation is increased. In morphine-insensitive tissues the output of noradrenaline per pulse may increase as much as 10-fold between 0.5 and 16 Hz (Hughes & Roth, 1974).

In the adrenergic system the major difference between morphine-sensitive and morphine-insensitive tissues is the relatively high noradrenaline output at low (< 1 Hz) frequencies of stimulation observed in morphine-sensitive tissues. A similar relationship between morphine sensitivity and a high transmitter output at low frequencies of stimulation has been observed in the cholinergic system (Greenberg *et al.*, 1970; Lees, Kosterlitz & Waterfield, 1972). It may be significant that in all morphine-sensitive tissues morphine has its greatest effect at low frequencies of stimulation.

It remains to be seen whether there is a causal relationship between the existence of functional opiate receptors and the frequency-output relationship of a particular neuronal system. Enkephalin, an endogenous ligand for opiate receptor sites (Hughes, 1975; Hughes, Smith, Morgan & Fothergill, 1975) also inhibits noradrenaline and acetylcholine release (Hughes, Kosterlitz & Waterfield, unpublished results) and is present in the guinea-pig ileum where it may have a functional role (Waterfield & Kosterlitz, 1975). It remains to be seen if enkephalin is present at other morphine-sensitive sites and thereby modulates neurotransmitter release.

Supported by grants from the Medical Research Council (to J. H.) and the U.S. National Institute on Drug Abuse (DA 00662) to Dr H.W. Kosterlitz. G.H. was a CASE scholar of the Science Research Council. Acknowledgement is made of generous gifts of drugs mentioned in the Methods section.

References

AMBACHE, N. & ZAR, M.A. (1971). Evidence against adrenergic motor transmission in the guinea-pig vas deferens. *J. Physiol., Lond.*, **216**, 359–389.

FURNESS, J.B., CAMPBELL, G.R., GILLARD, S.M., MALMFORS, T., COBB, J.L.S. & BURNSTOCK, G. (1970). Cellular studies of sympathetic denervation produced by 6-hydroxydopamine in the vas deferens. *J. Pharmac. exp. Ther.*, **174**, 111–122.

FURNESS, J.B. & IWAYAMA, T. (1971). Terminal axons ensheathed in smooth muscle cells of the vas deferens. *Z. Zellforsch.*, **113**, 259–270.

GREENBERG, R., KOSTERLITZ, H.W. & WATERFIELD, A.A. (1970). The effects of hexamethonium, morphine and adrenaline on the output of acetylcholine from the myenteric plexus-longitudinal muscle preparation of the ileum. *Br. J. Pharmac.*, **40**, 553P.

HENDERSON, G. (1974). The effects of morphine on peripheral adrenergic neuro-effector transmission. Ph.D. Thesis, University of Aberdeen.

HENDERSON, G. & HUGHES, J. (1975). Modulation of frequency-dependent noradrenaline release by calcium, angiotensin and morphine. *Br. J. Pharmac.*, **52**, 455P.

HENDERSON, G., HUGHES, J. & KOSTERLITZ, H.W. (1972). A new example of a morphine-sensitive neuro-effector junction: adrenergic transmission in the mouse vas deferens. *Br. J. Pharmac.*, **46**, 764–766.

HENDERSON, G., HUGHES, J. & KOSTERLITZ, H.W. (1975). The effects of morphine on the release of noradrenaline from the cat isolated nictitating membrane and the guinea-pig ileum myenteric plexus-longitudinal muscle preparation. *Br. J. Pharmac.*, **53**, 505–512.

HENDERSON, G. & NORTH, R.A. (1976). Depression by morphine of excitatory junction potentials in the vas deferens of the mouse. *Br. J. Pharmac.*, (in press).

HUGHES, J. (1972). Evaluation of the mechanisms controlling the release and inactivation of the adrenergic transmitter in the rabbit portal vein and vas deferens. *Br. J. Pharmac.*, **44**, 472–491.

HUGHES, J. (1973). Inhibition of noradrenaline release by lysergic acid diethylamide. *Br. J. Pharmac.*, **49**, 706–708.

HUGHES, J. (1975). Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine. *Brain Res.*, **88**, 295–308.

HUGHES, J., KOSTERLITZ, H.W. & LESLIE, F.M. (1975). Effect of morphine on adrenergic transmission in the mouse vas deferens. Assessment of agonist and antagonist potencies of narcotic analgesics. *Br. J. Pharmac.*, **53**, 371–381.

HUGHES, J. & ROTH, R.H. (1974). Variation in noradrenaline output with changes in stimulus frequency and train length: role of different noradrenaline pools. *Br. J. Pharmac.*, **51**, 373–382.

HUGHES, J., SMITH, T., MORGAN, B. & FOTHERGILL, L. (1975). Purification and properties of enkephalin — the possible endogenous ligand for the morphine receptor. *Life Sci.*, **16**, 1753–1758.

JENKINS, D.A., MARSHALL, I. & NASMYTH, P.A. (1975). Is noradrenaline the motor transmitter in the mouse vas deferens? *J. Physiol., Lond.*, **254**, 49–50P.

JONES, M.E.L. & SPRIGGS, T.L.B. (1975). Noradrenaline and motor transmission in the vas deferens of the mouse. *Br. J. Pharmac.*, **53**, 323–331.

LANE, B.P. & RHODIN, J.A.G. (1964). Cellular interrelationships and electrical activity in two types of smooth muscle. *J. Ultrastruct.*, **10**, 470–488.

LEES, G.M., KOSTERLITZ, H.W. & WATERFIELD, A.A. (1972). Characteristics of morphine-sensitive release of neurotransmitter substances. In *Agonist and Antagonist Actions of Narcotic Analgesic Drugs*, ed. Kosterlitz, H.W., Collier, H.O.J. & Villarreal, J.E., pp. 142–152. London: Macmillan.

MONTEL, H., STARKE, K. & WEBER, F. (1974). Influence of morphine and naloxone on the release of noradrenaline from rat brain cortex slices. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **283**, 357–369.

NORTH, R.A. & HENDERSON, G. (1975). Action of morphine on guinea-pig myenteric plexus and mouse vas deferens studied by intracellular recording. *Life Sci.*, **17**, 63–66.

O'HANLON, J.P., CAMPUZANO, H.C. & HORVATH, S.M. (1970). A fluorimetric assay for subnanogram concentrations of adrenaline and noradrenaline in plasma. *Analyt. Biochem.*, **34**, 568–581.

WATERFIELD, A.A. & KOSTERLITZ, H.W. (1975). Stereospecific increase by narcotic antagonists of evoked acetylcholine output in guinea-pig ileum. *Life Sci.*, **16**, 1787–1792.

YAMAUCHI, A. & BURNSTOCK, G. (1969). Post-natal development of the innervation of the mouse vas deferens. A fine structural study. *J. Anat.*, **104**, 17–32.

(Received January 23, 1976.)