plate test. Male rats, about 250 g, were used in groups of 5. All were conditioned to the hot plate (60 °C) by more than four exposures. Those jumping from the hot plate after 10 s or before 2 s were not used. The opiates were administered by injection into the cerebroventricles and an equal amount of 0·9% NaCl solution was also given to the control group. The reaction time on the hot plate was determined at various times up to 150 min after the injection to assess the analgesic activity.

Me-enk-ol at a dose of 0·05 mg kg^{-1} gave a peak analgesia (20 s) between 20 and 40 min falling away to a 10 s response at 90 min. The peak analgesia for morphine, 0·1 mg kg^{-1}, was observed between 10 and 120 min with a falling away to 17 s at 150 min. Replacement of the glycine at position 2 of Me-enk-ol with D-alanine increased the analgesic activity. MeTyr-D-Ala-Gly-Phe-Metol which is about 1/5 as active as Me-enk-ol in inhibiting the guinea-pig ileum twitch response was more potent than morphine and Me-enk-ol in the analgesic activity. The peak effect persisted for over 150 min at a dose of 0·05 mg kg^{-1} with a fall to 17 s at 170 min. Besides analgesia, catalepsy was seen after treatment with these peptides.

From the present experiments, it is concluded that *N*-methylation of the tyrosine residue and conversion of the methionine residue of methionine enkephalin to methioninol dramatically increased morphine-like activity in vitro. The results also indicate that replacement of the glycine at position 2 of Me-enk-ol with D-alanine increased the analgesic activity.

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Effect of cocaine on contractile responses to noradrenaline of canine isolated mesenteric vein

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The canine anterior mesenteric vein has an inner circular band of muscle and an outer layer of longitudinal muscle bundles (O'Connor & Slater 1972). A two-layer mesenteric vein occurs in other mammals (Loizou & Tindall 1969). It is possible to record the contractions of the muscle layers using ring, helical and longitudinal vein preparations (Sutter 1965; McConnell & Roddie 1970) but these inevitably disrupt the normal relation between the two layers. The contractions of two venous muscle groups can be measured independently by perfusing a vein segment and recording its longitudinal tension. The perfusion pressure monitors the circular muscle contractions and the isometric tension measures the longitudinal muscle contractions (Williamson 1969; Hall & O'Connor 1973).

The contractile activity of noradrenaline in isolated vascular tissue is affected by its uptake into sympathetic nerve terminals (de la Lande et al 1967). Application of noradrenaline (NA) to the intimal and adventitial surfaces often results in differences in the contractile response (de la Lande et al 1966, 1967; Crotty et al 1969).

This paper describes a method for adding noradrenaline to either surface of the canine mesenteric vein whilst recording the contractions of the circular and longitudinal muscles. Veins treated with cocaine were used to determine the extent to which neuronal uptake of noradrenaline affects the response of the two muscle layers.

A 5–8 cm segment of anterior mesenteric vein was removed from mongrel dogs of either sex, 10–20 kg, after death, the tributaries tied off, and all excess tissue was removed. Two circular tissue-baths, one inside the other, within a water-filled jacket at 37 °C, were used and the vein was mounted vertically in the inner bath. A fixed cannula at the base of the bath was inserted into the proximal end of the vein. An inverted

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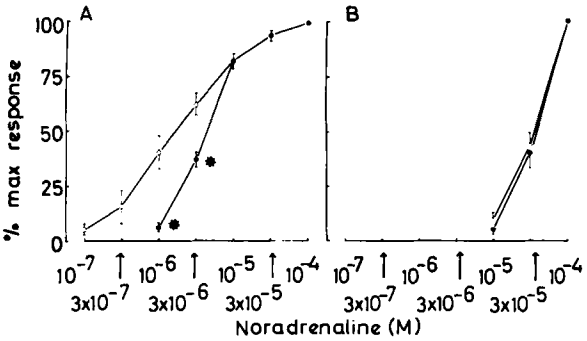

FIG. 1. The mean responses of A circular and B longitudinal muscle obtained by adding cumulative doses of NA (●——●), and NA plus 3×10^{-5} M cocaine (○——○) to A: the intraluminal and B: the extraluminal surfaces of 8 canine anterior mesenteric veins. Vertical bars represent s.e. Comparisons of cocaine treated with normal using a *t*-test: $*P < 0{\cdot}05$.

U-shaped tube was tied into the distal end of the vein which was perfused with Tyrode solution gassed with 5% CO_2 in oxygen, in a direction (proximal to distal) retrograde to normal flow by a Watson-Marlow constant flow pump. The pulsatile output of the pump was converted by an air reservoir into an almost linear flow. The fluid output from the lumen of the vein entered the U-tube emptying into the outermost tissue bath from which it was continuously recirculated through the cannula, so the outer bath fluid was pumped through the lumen of the vein, and the inner bath fluid was in contact with the outer surface of the vein. The upper end of the vein and the U-tube cannula were suspended from an isometric tension transducer to record changes in longitudinal muscle tone. Changes in perfusion pressure were monitored with a pressure transducer connected by a T-piece inserted between the pump and the vein. The outputs from the transducer were amplified and displaced on a pen recorder.

The preparation was allowed to equilibrate for 2 h in the Tyrode solution which was changed every 15 min. The tension on the longitudinal muscle was set at 2 g and the constant flow pump adjusted to provide a resting perfusion pressure of 10 mmHg. Preliminary experiments had shown that optimum responses were obtained under these conditions.

The vein was tested for transmural leaks at the beginning and during the experiments by allowing the constant flow pump to pass air into the lumen of the vessel while the outflow was restricted. Any leaks were ligated.

A total of 16 veins, 8 of which were pretreated for 1 h with cocaine (3×10^{-5} M), to inhibit the neuronal uptake of NA (Trendelenburg 1963), were exposed to a supramaximal concentration of NA (10^{-4} M) applied to each surface in turn. The inner circular muscle, represented by the perfusion pressure, was more sensitive to intraluminal NA than to extraluminal amine, whereas the outer longitudinal muscle response, represented by the tension, was independent of the route of administration (Table 1). Cocaine had no effect on contractions produced by the supramaximal NA concentrations. This permits the contractions produced by cumulative doses of NA to be expressed as a percentage of the maximum response.

Cumulative doses of NA (10^{-7} to 10^{-4} M) were added to 8 veins and the effects on the circular muscle were recorded. The mean dose-response curves obtained by adding NA to the two surfaces of the veins and recording changes in perfusion pressure are shown in Fig. 1. The circular muscle was highly sensitive to intraluminal NA and much less sensitive to extraluminal catecholamine. Cocaine pretreatment (1 h) significantly potentiated NA added intraluminally without affecting the maximum response, but had no effect on extraluminal NA.

The increases in longitudinal muscle tension produced by noradrenaline (10^{-7} to 10^{-4} M) added to the inner and outer surfaces of 8 mesenteric veins were recorded. The mean cumulative dose-response curves are shown in Fig. 2. NA applied to the outer surface of the vein caused a greater contraction of the longitudinal muscle than intraluminal NA. The responses to intra- and extraluminal noradrenaline were potentiated by cocaine pretreatment. A greater potentiation of the intraluminal NA was recorded, with the result that cocaine abolished the difference between intra- and extraluminal NA.

The potentiation of NA by cocaine was measured as the log-dose ratio (Table 2), the ratio of the logarithmic concentrations of NA required to produce 50% of the maximum response in the presence and absence of cocaine (Foster 1967). Cocaine potentiated noradrenaline, reaching the circular muscle from the inside of the vein but not from the outside. In contrast, cocaine potentiated noradrenaline reaching the longitudinal muscle from either vein surface. Statistical analysis (paired Student's *t*-test) showed that intraluminal NA was potentiated more than extraluminal monoamine (Table 2).

Table 1. The effects of NA alone in the presence of cocaine on the perfusion pressure and longitudinal tension recorded in 8 isolated canine mesenteric veins. Veins were pretreated with cocaine for 1 h before adding NA to the intra- and extraluminal surfaces. The perfusion pressure reflects the activity of the circular muscle and the tension measures the longitudinal muscle contractions.

Treatment (M)	Mean increase in perfusion pressure (mm Hg ±s.e.) Intraluminal NA	Extraluminal NA	Mean increase in tension (g ±s.e.) Intraluminal NA	Extraluminal NA
NA (10^{-4})	10·7 ± 0·7	5·5 ± 0·4*	32·0 ± 1·1	33·6 ± 1·2
NA (10^{-4}) + cocaine (3×10^{-5})	10·7 ± 0·9	5·5 ± 0·6*	31·6 ± 0·5	33·7 ± 0·9

Table 2. Cocaine potentiation of NA expressed as the log-dose ratio (Foster 1967) in anterior mesenteric vein preparations.

Log-dose ratios for circular muscle*	Log-dose ratios for longitudinal muscle†	
Intraluminal NA	Intraluminal NA	Extraluminal NA
0·80	1·0	0·52
0·35	1·75	0·74
0·83	1·10	0·83
0·63	0·55	0·32
0·38	1·10	0·41
0·44	1·25	0·30
0·29	1·15	0·61
0·36	1·40	0·52
0·51 ± 0·07	1·16 ± 0·11	0·53 ± 0·06

* Only the responses of the circular muscle to intraluminal NA were potentiated by cocaine (3×10^{-5} M).

† The responses of the longitudinal muscle (mean ± s.e.m.) to intraluminal and extraluminal NA were potentiated by cocaine (3×10^{-5} M). The degree of potentiation of the intraluminal NA was significantly greater than the extraluminal NA ($P < 0.05$).

Cocaine potentiates NA by inhibiting its neural uptake (Furchgott et al 1963; Iversen 1967) thus increasing its effective concentration at the receptors (Trendelenburg et al 1970; Granata & Langer 1973). This process, termed Uptake$_1$, is mainly responsible for terminating the actions of noradrenaline (Iversen 1967, 1971) whereas extraneuronal uptake (Uptake$_2$) has little influence on the cardiovascular actions of noradrenaline (Brown et al 1979). It has been proposed that cocaine also potentiates noradrenaline by a post-junctional action involving α-adrenoceptors (Kalsner & Nickerson 1969; Kalsner 1974; Greenberg & Long 1974; Greenberg & Innes 1976). A pre-junctional action is seen as a horizontal shift of the dose-response curve whereas a post-junctional effect increases the maximum response (Kalsner 1974). We have found that cocaine has no effect on the supramaximal dose of noradrenaline. This eliminates the possibility of post-junctional potentiation.

The potentiation of noradrenaline by inhibition of Uptake$_1$ depends on the extent of the sympathetic innervation (Trendelenburg et al 1969; Greenberg & Long 1974) and the neuromuscular junction interval (Verity 1971). Noradrenergic nerve terminals are distributed unevenly in the two muscle layers of the dog mesenteric vein and this led to a previous suggestion that cocaine potentiates NA in the circular muscle more than in the longitudinal muscle because the latter has the more sparse sympathetic innervation (Hall & O'Connor 1973). However, we have now shown that the potentiation by cocaine of NA in circular muscle occurs only when NA is added to the intimal surface. A similar, but less striking effect was recorded using the longitudinal muscle. Two alternative explanations are proposed, either the route of administration influences NA uptake (de la Lande & Waterson 1967) or the smooth muscle response is influenced by a mechanism that is independent of Uptake$_1$. The structure of the mesenteric vein is such that NA applied intraluminally first has to cross the circular muscle before reaching the outer longitudinal layer. Cocaine, by inhibiting Uptake$_1$ in the circular muscle, would therefore be expected to increase the amount of NA reaching the longitudinal muscle. This hypothesis is consistent with the present findings because the potentiation of NA by cocaine was much greater when NA reached the longitudinal muscle from the inside of the vessel than from the outside. We conclude that uptake of noradrenaline by nerve terminals in the circular muscle explains why intraluminal noradrenaline normally has a smaller effect on the longitudinal muscle than extraluminal catecholamine. Cocaine, by preventing Uptake$_1$ in the circular muscle, removes the directional-dependent action of noradrenaline on the longitudinal muscle. In contrast, the properties of the circular muscle of the vein cannot be explained entirely by the activity of the neuronal uptake system. Although cocaine potentiated intraluminal NA, which is consistent with the greater sympathetic terminal density in the circular layer, it had no effect on extraluminal NA. Thus, cocaine failed to abolish the directional property of NA on the circular muscle. The very low sensitivity of the inner circular layer to NA cannot therefore be explained by it being taken up by nerve terminals as it passes through the outer longitudinal muscle. These findings are in direct contrast to those of de la Lande & Waterson (1967) who perfused rabbit ear arteries and demonstrated that cocaine potentiated extraluminal much more than intraluminal NA. The ear artery does not have an outer longitudinal muscle layer and to explain their findings, de la Lande & Waterson proposed that the NA uptake sites were distributed around the outer perimeter of the circular muscle, so the amount of extraluminally applied NA would be reduced before it reached the receptors. Perhaps some morphological difference between veins and arteries accounts for the discrepancy. However, a study using the splenic artery, which has the usual circular muscle layer and therefore resembles the ear artery, has shown that cocaine does not prevent intraluminal NA from causing a much greater constriction than the extraluminal NA (O'Connor & Slater 1981). Thus, the hypothesis of de la Lande & Watersen (1967), based on the distribution of uptake sites, cannot be extrapolated to veins and other arteries.

An alternative mechanism to account for the directional-dependent responses to NA might involve the mechanisms by which it initiates and propagates smooth muscle contractions. Two mechanisms for the contractile responses of blood vessels have been proposed, a fast, propagated response and a slow, equilibrium type, non-propagated response (Bevan et al 1973), inferring that the way in which NA initiates the two mechanisms could be influenced by the route of admin-

istration, especially since it penetrates blood vessel walls non-uniformily (Török & Bevan 1971). Another factor contributing to the greater efficacy of intraluminal noradrenaline, could be non-uniformity of α-adrenoceptors in the vascular muscle which enables NA to pass from the intima to the receptors more easily.

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Effect of cocaine on noradrenaline contractions of mesenteric vein: pre- or postjunctional mechanism?

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Cocaine potentiates noradrenaline (NA) in tissues with a sympathetic innervation by inhibiting neuronal uptake (Macmillan 1959; Iversen 1967). However, a second, postjunctional, mechanism for this potentiation has been demonstrated (Kalsner & Nickerson 1969a; Kalsner 1974). It has been shown, with the spleen, trachea, nictitating membrane, rabbit ear artery and aorta, that the role of pre- and postjunctional mechanisms in relation to the potentiation of noradrenaline by cocaine is not uniform (Hertting et al 1961; de la Lande & Waterson 1967; Foster 1967, 1969; Reiffenstein 1968; Kalsner & Nickerson 1969b; Levin & Furchgott 1970; Draskoczy & Trendelenburg 1970; Innes & Karr 1971; Granata & Langer 1973; Kalsner 1974). Although the relative importance of the two mechanisms also varies among different vascular beds (Varma & McCullough 1969; Davidson & Innes 1970; Innes & Karr 1971; Granata & Langer 1973), very little is known about the potentiation of NA by cocaine in individual vessels, particularly veins.

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We have examined the effect of cocaine on the contractions produced by NA in the guinea-pig isolated portal mesenteric vein. Prejunctional uptake of NA in relation to cocaine was determined by pretreating guinea-pigs with 6-hydroxydopamine (6-OHDA) to produce a sympathetic chemical denervation of the tissue (Thoenen & Tranzer 1968).

Normal (control) and 6-OHDA pretreated guinea-pigs (300–500 g) of either sex were used. 6-OHDA hydrochloride was administered under ether anaesthesia. A femoral vein was exposed and 6-OHDA (250 mg kg^{-1}), freshly dissolved in 0·9% NaCl containing 0·2 mg kg^{-1} of ascorbic acid, was administered intravenously. The incision was infiltrated with a local anaesthetic and sutured. The animals were killed after 24 h, and a ligature was tied round the portal vein close to the liver and the vein traced to the duodenum, which was transected and retracted. A second ligature was applied and 2–2·5 cm of portal and anterior mesenteric vein was isolated. The vein was mounted in a tissue bath containing oxygenated Tyrode solution at 37 °C. One end of the vein was secured and the free end was attached to a calibrated, isometric transducer to record longi-