The exact mechanism of persistent localized decrease in uptake of thiamine radioactivity induced by morphine in brain stem of the chronically-morphinised rats is not known at the present time. However, in view of the active transport of thiamine into the neurones[9], an interference by morphine with the transport carrier could produce such a decrease, even though there may be sufficient thiamine in blood to support normal cerebral function. Mobilization of thiamine from axonal membrane by morphine, persistence in rat brain of potent agonists e.g. morphine[10], methadone[11,12], levorphanol[13] which have high tolerance and physical dependence liability; lack of persistence of thebaine[14], without such a liability may possibly be other contributing factors in the long-lasting decrease in uptake of thiamine radioactivity induced by morphine in the brain stem.

In view of the measurements of total thiamine radioactivity in these studies, it is not possible to state whether thiamine or its di or triphosphate metabolite(s) is involved. As thiamine or cocarboxylase neither produced detectable analgesia in rats[15] nor any effect on isolated intestine or guinea-pig uterus[16,17], our findings may not be related in a primary way to thiamine. There is evidence however, that thiamine decreased the intensity of morphine withdrawal syndrome in rats[18] and daily injections of thiamine at first prevented and afterwards delayed the progressive appearance of tolerance to morphine analgesia[15]. Hyperexcitability and aggressive behaviour normally seen in chronically-morphinized rats did not occur on injections of thiamine in the rats and the rate of recovery of morphine analgesia was also accelerated by these injections[15]. Thiamine therefore, may play an indirect role in opiate effects described above. Morphine-induced thiamine depletion in brain stem and loss of membrane-bound calcium in the CNS previously reported[19] with morphine could conceivably cause an abnormality in the role of thiamine[3,4], in maintaining an essential configuration of the sodium transport system of the excitable membranes and consequent changes in ion transport may have an important bearing on tolerance to and physical dependence on morphine.

Finally our results with opiates appear interesting in view of the fact that single doses of amobarbital (25 mg/kg i.p.) or ethanol (5 g/kg p.o.) in rats did not produce any significant change in incorporation of thiamine in any areas of the CNS or plasma. Intravenous injection (5 mg/kg) of cocaine however, produced a significant increase ($P<0.05$) as compared to the saline controls in the incorporation of labelled thiamine radioactivity in the cortical hemispheres (12.4%), cerebellum (17.3%) and brain stem (14.7%) but not in plasma of rats (Misra et al., unpublished observations).

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Inhibitory effect of methionine- and leucine-enkephalin on contractions of the guinea-pig ileum elicited by PGE_1

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Summary. Methionine-enkephalin and leucine-enkephalin (m-enk and l-enk) have been shown to antagonize contractions of the isolated guinea-pig intestine elicited by PGE_1. The inhibitory effect of these 2 pentapeptides is abolished by naloxone.

Recently, the existence of an endogenous substance in the brain, that acts as an agonist at opiate-receptor sites, has been reported[1–3]. Hughes et al. characterized this substance, which they named enkephalin as a low-molecular-weight peptide[4]. Enkephalin has since been found to consist of 2 pentapeptides, methionine-enkephalin and leucine-enkephalin (hereafter abbreviated to m-enk and l-enk, respectively), which have been identified and synthesized[5]. Both have been shown to produce a dose-related inhibitory effect on electrically evoked contractions of the mouse vas deferens and the guinea-pig ileum[5,6]. The same authors noted some slight quantitative differences between the 2 peptides, l-enk being on the whole somewhat less active than m-enk. They also demonstrated that naloxone reverses the depressant effects of m-enk and l-enk on electrically induced contractions of the vas deferens of the mouse. Chang et al.[7] have studied the opiate-receptor affinities of synthetic m-enk and l-enk and have found that:

a) m-enk has slightly greater affinity for opiate-receptors in the rat brain than l-enk, and

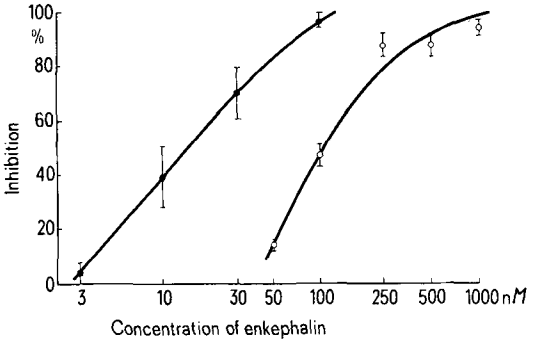
b) m-enk injected intracerebrally produces a transient analgesia that is reversed by naloxone. When m-enk and l-enk were administered through permanently indwelling cannulae in the lateral ventricles of rats, both were found to induce a profound analgesia that was completely abolished by naloxone[8].

We have shown that morphine and other narcotic analgesic agents inhibit the contractions elicited by prostaglandin E_1 in the isolated guinea-pig ileum[9], and that by using this simple in vitro technique, it is possible to distinguish between narcotic agonist and antagonist activities of drugs[10]. It therefore seemed possible that enkephalins might also antagonize intestinal contractions elicited by PGE_1 and, if so, that this inhibitory effect would also be reversible through the interaction of an opiate antagonist, viz. naloxone. The following experiments were carried out to test this hypothesis.

Materials and methods. Methionine- and leucine-enkephalin were synthesized by Dr M. Rittel of our Chemistry Department. Prostaglandin E_1 was purchased from Ono Pharmaceutical Company, Ltd, Osaka (Japan), and naloxone hydrochloride from Endo Laboratories Inc., Garden City, N.Y. (USA). Toloconium methylsulphate (active principle of Desogen®) was obtained from our Chemistry Department.

Segments (length of 5–6 cm) of terminal ileum (excluding the 10-cm-portion next to the ileocoecal valve) from male guinea-pigs (Pirbright strain; Tierfarm AG, Sisseln; body weight between 350 and 550 g) were mounted in a 20 ml organ-bath containing Tyrode's solution maintained at 38 °C and aerated with O_2. Usually, 1–2 h elapsed before the experiments were started. Following a recommendation made by J. H. Gaddum[11], we used a detergent to prevent the pentapeptides from being adsorbed on to glass and other foreign surfaces. One drop of a 0.1% (w/v) toloconium methylsulphate (toloconium) solution in distilled water was added to 10 ml stock solutions (0.1 to 1 mM) of the pentapeptides. The stock solutions were prepared less than 1 h before the in vitro experiments began. Further dilution of these solutions was always preceded by the addition of one drop of a 0.1% toloconium solution. The final concentration of toloconium in the organ-bath never exceeded 0.001%. The concentrations of PGE_1 used in this study ranged from 0.1 to 0.3 µg/ml, 0.1 µg/ml being the most frequently used dose. Intestinal segments which did not respond in a reproducible manner to PGE_1 were discarded.

Results. As can be seen from the figure, very low concentrations of both enkephalins produced a dose-dependent inhibition of the contractions elicited by PGE_1 in the isolated guinea-pig ileum. The enkephalin concentrations active under these experimental condition are almost identical with those reported to inhibit electrically evoked contractions of the guinea-pig ileum[5]. The specific narcotic antagonist naloxone was found to reverse the inhibitory effects of the enkephalins at concentrations of 650 to 1000 mM, i.e. concentrations similar to those quoted in the above-mentioned paper. A chance observation deserves mention in this connection: Under the given experimental conditions, the ileum of the guinea-pig usually displays no spontaneous activity or increase in tone. In the present series of experiments, spontaneous contractions of the longitudinal and circular muscle and increases in tone were nevertheless observed in a few segments. In these cases, the addition of small amounts of either enkephalin (10–30 mM) resulted in a very rapid cessation of the contractile activity and total relaxation of the intestine.



Inhibitory effect of methionine-enkephalin (shaded circles) and leucine-enkephalin (circles in outlines) on contractions of the isolated guinea-pig ileum elicited by PGE_1. Each plot represents the mean of 5 determinations ± SD.

From the findings published by other authors concerning the various opiate-like activities of enkephalins and from the results obtained in the present study, certain interesting general features emerge. It is obvious that the enkephalins exert actions similar to those of morphine and narcotic analgesics, and their repeated administration likewise leads to development of tolerance[12] and dependence[13]. Like morphine and other narcotic analgesics, enkephalins inhibit the effect of PGE_1 on smooth muscle. It would therefore seem that prostaglandins are implicated at some point in the pathogenesis of pain. This view is corroborated by findings recently described by Lembeck et al.[14], who also quote relevant data published by other authors. Although they have not yet been demonstrated in the intestine, it is tempting to speculate that enkephalins or enkephalin-like peptides could occur in this organ, for example in peptidergic neurones of the mesenteric plexus. Such a view is supported by the following findings: a) 'the relative affinity of drugs (opiates) and the degree of stereospecificity for intestinal binding sites are closely similar to these properties in the brain'[6]; b) that enkephalins exert opiate agonist activity when administered into the brain[8,15] but also interact with opiate receptors in brain homogenates, the guinea-pig ileum and the mouse vas deferens[16]; and c) the recent isolation from human blood of a peptide (anodynin) that has been shown to interact with opiate receptors in brain homogenates and to cause analgesia when injected intracerebrally[17]. These various observations could indicate that peptides with agonist activity might be present in the intestine. If this were so, it would be conceivable that an interaction between prostaglandins and enkephalins could play an important role in regulating gastro-intestinal motility. Normal physiological gastro-intestinal motility could reflect a state of balance between prostaglandin and enkephalin production; constipation, on the other hand, could be due to exaggerated enkephalin production or a deficiency in PGE_1 production, or both, and diarrhoea might result from exaggerated PGE_1 production or a decrease in the formation and release of enkephalin.

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