synaptic cleft, i.e. within the intrasynaptic region. There is experimental evidence that such a gradient does in fact exist in this tissue during neurogenic activity[5]. This suggests that only the smooth muscle cells directly adjacent to the nerve plexus and possibly only the post-synaptic membrane contain alphareceptors.

Accumulation and metabolism of ^{3}H-1-norepinephrine in rat portal vein*

Condition	Incubation time (min)	^{3}H-1-NE + ^{3}H-metabolites** (moles/g tiss) $\times 10^{-10}$ (mean ± SE [n])
a) Control	20	87.1 ± 5.0 (5)
b) Cocaine (10^{-4} M)	20	35.6 ± 4.1 (5)
Cocaine-sensitive (a–b)	20	49.3 ± 2.0 (5)
c) Control	40	200.3 ± 14.1 (5)
d) Cocaine (10^{-4} M)	40	87.9 ± 9.9 (5)
Cocaine-sensitive (c–d)	40	112.3 ± 6.7 (5)
		(moles/g min) $\times 10^{-10}$
Rate of cocaine sensitive accumulation and metabolism of ^{3}H-1-NE		3.15 ± 0.33

* Portal veins incubated in ^{3}H-1-NE (2×10^{-7} M); tissues assayed for ^{3}H-1-NE and ^{3}H-metabolites; Krebs medium assayed for ^{3}H-metabolites.

** Metabolites: 3,4-dihydroxyphenylglycol, 3-methoxy-4-hydroxyphenylglycol, 3,4-dihydroxymandelic acid, 3-methoxy-4-hydroxymandelic acid, normetanephrine.

Recently, Aprigliano et al.[10,11] have reported only a 1.6fold shift of the contractile dose-response curve of rat portal vein to exogenous NE after treatment in vitro with 6-hydroxydopamine. Hypersensitivity of this order is commonly seen with other blood vessel strips, and implies a more homogeneous distribution of the alpha-receptors throughout the longitudinal smooth muscle layer. This value is consistent with the dimensions of the calculated intra-mural NE gradient found in this study. However, it has also been shown[4,7,8] that the latency of onset of the contractile response to exogenous NE diffusing in from either the intimal or adventitial border is consistent with alpha-receptor localization near the adrenergic nerve plexus and between the smooth muscle layers. In addition, the portal vein longitudinal smooth muscle is capable of myogenic conduction which could effectively excite smooth muscle cells throughout the longitudinal muscle layer from layers near the nerve plexus. Its contractile activity is usually dominated by pacemaker cells and the concentration of neurogenic NE outside the synaptic cleft is probably too low to be biologically effective during physiological rates of sympathetic activity[5]. Thus, the tissue seems to possess a true neuroeffector junction. In other tissues with such a junction there is restriction of the receptors to the post-synaptic membrane.

In conclusion, evidence has been derived from studies of neuronal uptake and metabolism of ^{3}H-1-NE that the concentration gradient of exogenous NE in the longitudinal muscle of the rat portal vein is not sufficient to account for a high level of denervation or prejunctional hypersensitivity. If this, in fact, occurs in this tissue, it must be the consequence of a high intrasynaptic concentration gradient and a restriction of the alpha-adrenergic receptors to this site.

Differential effects of opiates on the incorporation of [^{14}C] thiamine in the central nervous system of the rat

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Summary. Opiate agonist (morphine), pure antagonist (naloxone), mixed agonist-antagonist (nalorphine) and analgesically inactive enantiomorph (dextrorphan) produced differential stereoselective effects on the incorporation of [^{14}C] thiamine in the central nervous system of the rats. The possible role of thiamine in opiate effects and its implications are discussed.

Thiamine and its phosphorylated esters appear to play an important biophysical role in nerve conduction and excitation at the molecular level[2,3], quite independently of their well-known coenzymatic activity in the decarboxylation of α-keto acids and 2 transketolation steps of the pentose phosphate pathway. The specific location of thiamine in the axonal membrane, rather than in the cytoplasm as in other cells, its ability to form complexes with sodium and calcium ions and some neurotransmitters, its release from several neural tissues on electrical stimulation and by drugs which interact with nerve membranes, appear consistent with its participation in neural excitation and transmembrane ion transport involving permeability changes at the sodium channel[4]. In view of the involvement of approximately half of the cerebral thiamine in pyruvate oxidation and the remaining in other metabolic processes in brain, there is little if any excess of thiamine in the CNS[5] and disturbance of function in the CNS with its moderate depletion is understandable.

This study demonstrates that in chronically-morphinised rats, a highly significant decrease as compared to the controls occurred in the incorporation of [thiazole-2-^{14}C]thiamine in the brain stem; concurrent administration of naloxone, the pure narcotic antagonist with morphine abolished the highly significant increase in the incorporation of thiamine radioactivity in cortical hemispheres, cerebellum, brain stem and plasma of rats acutely-treated with a 10 mg/kg s.c. dose of morphine; dextrorphan, an analgesically-inactive morphinan did not produce any significant change in incorporation of thiamine radioactivity in these areas of the CNS and plasma; naloxone produced a significant decrease in the incorporation of thiamine radioactivity only in the brain

stem. The biological implications of these observations are discussed here.

Materials and methods. [Thiazole-2-^{14}C]thiamine, sp. act. 41.7 μCi/mg and NCS solubilizer were obtained commercially from Amersham-Searle Corp., Illinois. Male Wistar rats (110–160 g) were used in these studies. Other details on methods are described in the legends to the figures 1 and 2.

Results and discussion. The data on the incorporation of [^{14}C]thiamine radioactivity in areas of the CNS and plasma of control, acutely and chronically-morphinized rats are given in figure 1. After a single 10 mg/kg s.c. injection of morphine, a highly significant ($p < 0.01$) increase as compared to the controls occurred in the incorporation of thiamine radioactivity in the cortical hemispheres (21%), cerebellum (44%), brain stem (29%) and plasma (53%) of the rats. In morphine pellet (75 mg)-implanted rats, 3 days after implantation, only cerebellum showed a significant increase (11%, $p < 0.05$) in incorporation of thiamine radioactivity as compared to the controls. However, one week after implantation, a significant decrease (9%, $p < 0.05$) appeared in the cortex and the brain stem (15%, $p < 0.01$) and 2 weeks after implantation the same significant decrease (15%, $p < 0.05$) persisted only in the brain stem.

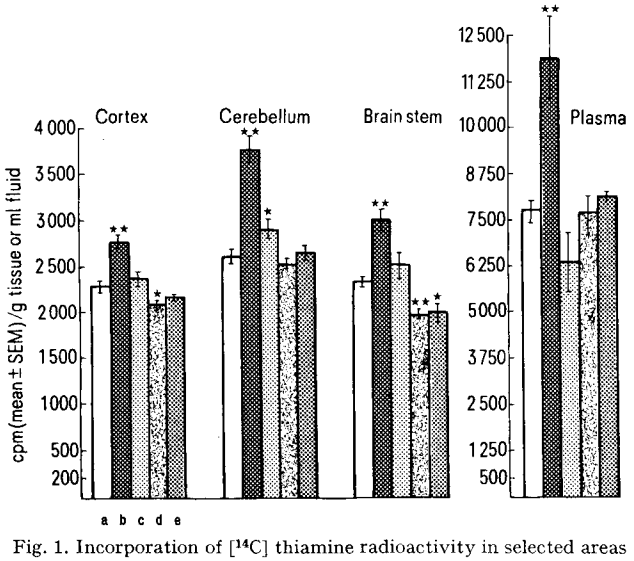

Fig. 1. Incorporation of [^{14}C] thiamine radioactivity in selected areas of the CNS and plasma of control, acutely and chronically-morphinised rats. The ordinate represents radioactivity as counts per min. (mean ± S. E. M.) per g wet tissue weight or ml plasma of 5 animals in each group. * and ** denote significant differences from control values at $p < 0.05$ and $p < 0.01$ respectively based on t-test. The rats in the control group (a) were injected s. c. with 0.9% saline (2 ml/kg); in the acute group (b) with a 10 mg/kg (free base) dose of morphine s. c. and in the chronic group a morphine pellet (75 mg) was implanted in each rat in the dorsal area. 15 min after injection of saline or morphine, [^{14}C] thiamine (1 mg/kg) was injected s. c. in each rat and blood and brain removed 30 min after this injection. Brain was dissected in different areas and blood immediately centrifuged to remove plasma. The tissue was homogenized in 0.9% saline to bring up to a total of 5 ml and an aliquot (0.5 ml) of homogenate in duplicate (plasma samples similarly treated) taken in counting vials, dissolved in 1–1.5 ml NCS solution at 45 °C on a Fisher-Slide Warmer and the radioactivity determined using 10 ml toluence-phosphor solution in a liquid scintillation counter using conventional procedures. Duplicate determinations were performed on each tissue or plasma sample and the results averaged. In the chronically-morphinized group, brain and blood were removed 3 days (c), 1 week (d) and 2 weeks (e) after the implantation of morphine pellet. [^{14}C] Thiamine injections were given at the end of these periods and 30 min later, tissue and plasma removed and processed exactly as described above. The morphine pellet remained in the dorsal area till the time of sacrifice. Cortex represents the cortical hemispheres.

The data on the incorporation of thiamine radioactivity in the above areas of the CNS and plasma of control rats and those after s.c. injections of dextrorphan (10 mg/kg), naloxone (10 mg/kg) and morphine-naloxone mixture (10 mg/kg dose for each component) are given in figure 2. No significant differences in the incorporation of thiamine radioactivity as compared to the controls occured in any areas of the CNS and plasma of rats with dextrorphan. Naloxone produced a significant decrease (16%, $p < 0.05$) in incorporation only in the brain stem. The s.c. injection of morphine-naloxone mixture abolished all the increases observed in the incorporation of thiamine radioactivity in areas of the CNS and plasma of rats acutely-treated with 10 mg/kg s.c. dose of morphine. Nalorphine, a mixed agonist-antagonist (10 mg/kg s.c.) alone produced small increases in the incorporation of thiamine radioactivity in cerebellum (5%), brain stem (7%) and plasma (18%) of rats ($n = 4$), which were statistically not significant.

These studies on the incorporation of thiamine radioactivity in the CNS showing differential stereoselective effects with opiate agonist, pure antagonist, mixed agonist-antagonist and inactive enantiomorph provide evidence that morphine interferes at the brain stem with the biological processes in which thiamine is involved and also indicate that the brain stem may be an important site of morphine analgesia[6–8] and narcotic antagonism.

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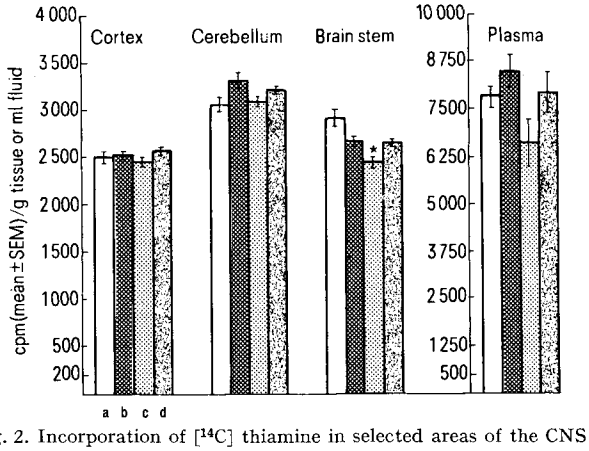

Fig. 2. Incorporation of [^{14}C] thiamine in selected areas of the CNS and plasma of rats (4 animals in each group) treated with saline (a), 10 mg/kg s.c. dose of dextrorphan (b), 10 mg/kg s.c. dose of naloxone (c) and 10 mg/kg s.c. dose each of morphine-naloxone in the same injection solution (d). * denotes significant differences from control values at $p < 0.05$ based on t-test. Other details as given in the footnote of figure 1.

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.