Analgesia following intravenous administration of enkephalin analogues

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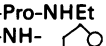
Methionine and leucine enkephalins, endogenous opiate peptides isolated from brain (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975), are not analgesic following intravenous administration to rodents, although both peptides are potent *in vitro*. It was considered that rapid enzymic inactivation was the most likely reason for the lack of *in vivo* activity (subsequently demonstrated by Hambrook, Morgan, Rance & Smith, 1976) and we have therefore sought to synthesize structural analogues which would have the required pharmacological properties to elicit analgesia *in vivo*. The analogues were assayed *in vitro* on the coaxially-stimulated guinea pig ileum preparation (Kosterlitz & Watt, 1968) and *in vivo* following intravenous administration in the mouse hot-plate test for analgesia (Eddy & Leimbach, 1953).

Table 1 gives the data obtained with a few of the analogues synthesized and indicates how activity has been improved from leucine enkephalin, which is inactive *in vivo*, to analogues which are analgesic at 5 mg/kg i.v. The favourable effect of D-amino acid substitution on opiate receptor binding affinity (Pert, Bowie, Fong & Chang, 1976) and analgesic potency following intracerebral injection (Baxter, Goff, Miller and Saunders, 1976; Pert, 1976) has already been reported but, as far as we are aware, only Bajusz and his colleagues have reported analgesia following intravenous administration of peptide analogues (Bajusz, Rónai, Székely, Gráf, Dunai-Kovács & Berzétei, 1977). We, too, have found many of our analogues to be active by this route and some are also active following s.c. or p.o. administration. Thus, ICI 120, 518 produced significant increases in reaction time for at least 90 min following administration of 25 mg/kg s.c. or 100 mg/kg p.o.

However, none of these enkephalin analogues is as active by the i.v. route as the potency on the ileum would suggest. One possible reason for this discrepancy is the relative inability of these peptides to cross the blood brain barrier. However, a comparison of the potency, relative to morphine, of a number of analogues in the mouse (hot-plate analgesia) and the 5 day-old chick (sedation) showed a similar potency in both species, indicative that a less obvious explanation for the relatively poor *in vivo* activity may have to be sought.

Table 1 Activity of enkephalin analogues

ICI No.	*Structure*	*Ileum**	*Mouse Hot Plate*	
			Dose (mg/kg i.v.)	*Reaction time (s)***
114,740	Tyr-Gly-Gly-Phe-Leu-OH	0.2	100	0
116,031	Tyr-Gly-Gly-Phe-Azleu-NH_2	0.6	100	+2
117,878	Tyr-D-Ala-Gly-Phe-Leu-OMe	5.6	100	+14
116,750	Tyr-D-Ser-Gly-Phe-Leu-OMe	11.0	10	+8
118,736	Tyr-D-Ser-Gly-Phe-Met-OMe	5.2	25	+4
120,518	Tyr-D-Ala-Gly-Phe-Pro-NHEt	0.9	5	+4
122,984	Tyr-D-Ser-Gly-Phe-Pro-NHEt	0.7	25	+10
121,444	Tyr-D-Ala-Gly-Phe-NH- 	3.2	5	+5

* Mean agonist potency relative to normorphine calculated from ID_{50}'s obtained on two preparations.
** Mean peak increase compared to pre-drug values following i.v. administration to groups of 3 mice. Mean increase with saline = 0.7 ± 0.2 s (mean ± s.e. mean, $n = 32$).

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Lack of serotoninergic involvement in turning behaviour induced by a unilateral lesion of the locus coeruleus in rats

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Unilateral lesions of the rat locus coeruleus produce circling behaviour apparently due to an asymmetry of striatal dopamine receptor activity (Donaldson, Dolphin, Jenner, Marsden & Pycock, 1976). Such circling may be mediated indirectly *via* the raphé nuclei since there is biochemical and histological evidence for a projection from locus coeruleus to the raphé system (Kostowski, Samanin, Bareggi, Marc, Garattini & Valzelli, 1974; Dahlstrom & Fuxe, 1964). We have investigated this possibility.

Ten days after a unilateral electrolytic lesion of locus coeruleus animals circled contraversively in response to apomorphine HCl (0.5 mg/kg, s.c.). At this time after surgery there was fall in ipsilateral forebrain noradrenaline (NA) levels ($P<0.05$), but ipsilateral dopamine (DA), 5-hydroxytryptamine (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) levels were unchanged ($P>0.05$).

Bilateral electrolytic lesions of locus coeruleus produced no obvious behavioural changes. Three and ten days following surgery, cortical NA levels were decreased bilaterally ($P<0.05$), forebrain DA and 5-HT levels were unchanged ($P>0.05$) but cortical and midbrain 5-HIAA levels were elevated ($P<0.05$). Pretreatment of animals at three days with *p*-chlorophenylalanine methyl ester HCl (200 mg/kg 36 h and 12 h prior to death) decreased striatal and mesolimbic 5-HT levels bilaterally in comparison to identically treated sham operated animals ($P<0.02$). This data provides evidence for increased 5-HT turnover following bilateral locus coeruleus lesions.

Pretreatment of animals with a unilateral locus coeruleus lesion at three days with α-methyl-*p*-tyrosine methyl ester HCl (200 mg/kg 18 h and 1.5 h prior to death) decreased cortical, mesolimbic and midbrain NA on the unlesioned side in comparison to identically treated sham operated animals ($P<0.05$). This data provides evidence for an increase in NA turnover in the contralateral forebrain, following a unilateral locus coeruleus lesion.

Electrolytic bilateral lesioning of the dorsal and median raphé nuclei and a unilateral lesion of locus coeruleus (on the same or a subsequent occasion) produced a bilateral fall in cortical, striatal, mesolimbic, hypothalamic and mid-brain 5-HT ($P<0.05$) and a unilateral fall in cortical and mesolimbic NA ($P<0.05$) ten days following surgery. These animals were hyperactive and showed spontaneous contralateral rotation.

This data indicates a bilateral input from locus coeruleus to the raphé nuclei that regulates 5-HT activity. However, a unilateral locus coeruleus lesion did not affect 5-HT turnover, and the resulting circling behaviour was not prevented by bilateral raphé nuclei lesions.

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