phine (5 ng/ml).‡ It is speculated that this lack of antagonist activity may be due to the lack of rigidity of the piperidine ring.

Substitution in the 6 position in addition to the 4-OH substitution e.g. ICI 97,628 (R=OH; R_1=Me; R_2=Cl) leads to compounds with sedative and antihistaminic properties together with opiate activity. The pharmacological properties of ICI 97,628 have been investigated in considerable detail and will be described in this communication.

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A microiontophoretic study of enkephalin and enkephalin analogues on brain stem neurones in the rat

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Following the identification and synthesis of met- and leu-enkephalin and the demonstration of their opiate agonist activity in a variety of test situations, it became apparent that the intensity and duration of their actions was limited by rapid inactivation in brain tissue. Several peptides have now been synthesized which appear to be more stable. For example, substitution of D-alanine for glycine, in the 2-position of met-enkephalin results in a peptide which retains its affinity for opiate receptors, but which resists inactivation *in vitro* (Pert, Bowie, Fong & Chang, 1976). This analogue produces long-lasting analgesia in the rat when administered intra-cerebrally (Pert, 1976). A similar analogue of leu-enkephalin, Tyr-D-ala-Gly-Phe-D-leu (Burroughs-Wellcome BW 180C) has been shown to have analgesic activity when injected intra-cerebroventricularly (Baxter, Follenfant, Miller & Sethna, 1977).

With the alterations to the structure of the enkephalins, it was of interest to see what effects these novel peptides exerted at single neurone level, and also to compare their actions with those of the endogenous compounds. We have therefore investigated the effects of these compounds, applied microiontophoretically, on single medullary neurones, and compared the actions with those of leu- or met-enkephalin and etorphine. Male Sprague-Dawley rats were used, anaesthetized with urethane (1.2–1.8 g/kg) and prepared as previously described for recording from the medulla (Bradley & Dray, 1974).

Drugs used were D-(ala)^2-met enkephalin amide (17 mM pH 5.7), D-(ala)^2-D-leu^5 enkephalin-HCl (16.5 mM pH 3.0), met-enkephalin (15 mM pH 5.0), leu-enkephalin (15 mM pH 4.5), etorphine-HCl (25 mM pH 5.0), naloxone-HCl (25 mM pH 4.5). Ejection of Pontamine Sky Blue dye was used in many experiments to mark the position of cells responding to a peptide and/or etorphine.

Etorphine and the peptides consistently produced depression of the spontaneous activity of the cells to which they were applied. These depressions could be antagonized by prior or concurrent administration of the opiate antagonist naloxone. However, while the response to the enkephalins was of short duration, that to the stable analogues was much longer, lasting up to 10 min after the end of application. The depression of firing rate produced by etorphine was also long-lasting, as previously reported (Bradley, Briggs, Gayton & Lambert, 1976).

One striking feature of these experiments was the high percentage of cells affected by the peptides—some 80–90%. When morphine was tested in a similar manner (Bradley & Dray, 1974; Bramwell & Bradley, 1974) a lower percentage of cells responded and a significant number of naloxone-insensitive excitations were seen.

For the two enkephalin analogues tested, the amino-acid sequence modifications appear to confer greater stability without producing any qualitative change in the neuronal responses. This is entirely consitent with a transmitter role for enkephalin since it suggests that a mechanism exists for the rapid inactivation of the endogenous peptides *in vivo*.

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In vivo effects of GABA uptake inhibitors on the response of cat spinal neurones to GABA analogues

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The 'high' affinity uptake of GABA by slices of cat spinal cord is inhibited by relatively low concentrations of (+)-2,4-diaminobutyric acid (DABA), (−)-nipecotic acid, guvacine and arecaidine. The importance of such carrier-mediated uptake into neurones and glia in terminating the action of GABA *in vivo* has been suggested by the finding that these inhibitors enhance the inhibition of neurones by electrophoretic GABA in the cat central nervous system (Lodge, Johnston, Curtis & Brand, 1977). The possibility was not excluded, however, that the inhibitors enhanced the action of GABA by interactions at postsynaptic GABA receptors and/or ionophores.

Muscimol and isoguvacine are potent bicuculline-sensitive depressants of the firing of cat spinal neurones. In cat spinal tissue muscimol is a weak inhibitor of GABA uptake (33% inhibition at 2×10^{-4} M; GABA 10^{-8} M) whereas isoguvacine does not influence uptake significantly at 2×10^{-4} M. Thus high affinity GABA uptake processes seem unlikely to be involved in terminating the action of these two compounds *in vivo*, particularly that of isoguvacine.

Experiments were performed on 6 cats anaesthetized with pentobarbitone to compare the effects of the inhibitors of GABA uptake on the inhibition of spinal neurones by electrophoretic GABA, muscimol and isoguvacine. The action of muscimol was enhanced to a similar extent to that of GABA on all 10 cells tested by concentrations of (−)-nipecotic acid (7 cells), DABA (3 cells) and arecaidine (3 cells) which did not enhance the action of glycine.

The action of isoguvacine on 16 of 21 cells was not increased by the uptake inhibitors ejected in amounts which enhanced the inhibitory action of GABA: (−)-nipecotic acid (10 cells), DABA (5 cells), guvacine (15 cells). When the currents ejecting the inhibitors were increased 3–4 fold the action of isoguvacine was also increased. On the other 5 cells the inhibitors increased the effect of isoguvacine, but to a lesser extent than that of GABA, even when ejected with relatively low currents.

The results with muscimol indicate that the uptake inhibitors may interact cooperatively at GABA postsynaptic receptors/ionophores, so enhancing the effectiveness of GABA agonists, and/or interfere with transport processes which inactivate muscimol. The selectivity demonstrated using GABA and isoguvacine might indicate that low concentrations of the uptake inhibitors enhance the action of GABA by a relatively specific effect on cellular uptake. On the other hand this selectivity may have arisen from differences in the tissue distribution of electrophoretically administered GABA and isoguvacine. More direct evidence is required of the nature of possible transport processes for muscimol and isoguvacine in spinal tissue.

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