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The xanthene-spiropiperidines: a new group of centrally-active drugs

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Any molecule which possesses a planar area (usually aromatic) and a nitrogen atom capable of occupying the same juxtaposition in space as the phenolic ring and the nitrogen of morphine can theoretically possess opiate properties. The Beckett and Casy model (Figure 1a) represents these requirements. From a variety of structures synthetized in a search for novel analgesic drugs, the xanthene-spiropiperidines of the general structure indicated in Figure 1(b) proved to be the most interesting. The similarity between the spiropiperidine moeity and morphine can be seen by comparison with Figure 1(c).

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The unsubstituted spiropiperidine (ICI 81,058: R and R_2=H; R_1=Me) is virtually devoid of opiate properties (activity relative to normoprhine = < 1% on the coaxially stimulated guinea pig ileum preparation [Paton (1957); Kosterlitz & Watt (1968)]), but is a potent sedative (54% decrease in locomotor activity of mice in photocell activity cages 1 h after 10 mg/kg p.o.) and antihistaminic (pA_2 v histamine on guinea pig ileum = 8.1). However, opiate properties are introduced when an hydroxyl is inserted at the 4 position (ICI 86,458: R=OH; R_1=Me; R_2=H). ICI 86, 458 has 16% of the potency of normorphine on the guinea pig ileum preparation and is analgesic (ED_{50} against acetic acid-induced writhing in mice = 1.3 mg/kg s.c.). The acetylated analogue (ICI 91,356: R=OAc; R_1=Me; R_2=H) is equipotent with normophine on the ileum and is a more effective analgesic (ED_{50} against acetic acid-induced writhing = 0.6 mg/kg s.c.).

Attempts to synthesize potent antagonists or partial agonists of the spiropiperidine series have met with little success e.g. the cyclopropylmethyl analogue (ICI 87,542: R=OH; R_1=CH_2-◁ ; R_2=H) has only 3% of the potency of naloxone on the mouse vas deferens preparation constantly perfused with etor-

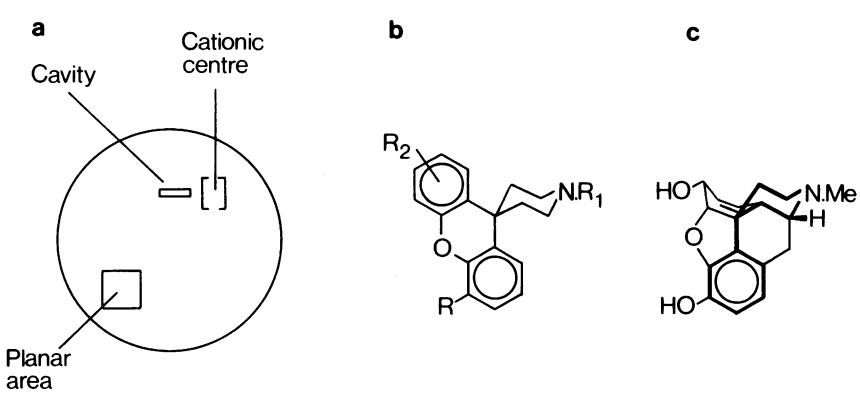

Figure 1 (a) Model of Beckett & Casy, 1954; (b) Spiropiperidine; (c) Morphine.

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*.

LAL is an MRC Student.