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# ASSESSMENT IN THE GUINEA-PIG ILEUM AND MOUSE VAS DEFERENS OF BENZOMORPHANS WHICH HAVE STRONG ANTINOCICEPTIVE ACTIVITY BUT DO NOT SUBSTITUTE FOR MORPHINE IN THE DEPENDENT MONKEY

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1 Four benzomorphans which have potent antinociceptive activity in the hot-plate and writhing tests in the mouse but do not suppress or precipitate withdrawal symptoms in the morphine-dependent monkey, have been examined for their pharmacological actions in the guinea-pig ileum and mouse vas deferens.

2 In the guinea-pig ileum their agonist potencies are 1.5 to 400 times greater than that of normorphine or morphine whereas in the mouse vas deferens their potencies relative to morphine are 0.3 to 100. They exhibit no antagonist activity in either preparation. Benzomorphans which substitute for morphine in the morphine-dependent monkey do not show such differences between their relative potencies in the guinea-pig ileum and mouse vas deferens.

3 The relative potencies of the four benzomorphans to inhibit stereospecific [$^3$H]-dihydromorphine binding by membrane fragments from rat brain, are more closely related to their relative agonist potencies in the mouse vas deferens than to those found in the guinea-pig ileum.

4 In order to antagonize the agonist actions of these benzomorphans, naloxone is required in concentrations which are 3 to 7 times higher than those needed for the antagonism of normorphine or morphine or of benzomorphans which suppress abstinence in morphine-dependent monkeys.

5 It may be possible to use the three assays, namely, ratio of relative agonist potency in mouse vas deferens to that in guinea-pig ileum, ratio of relative agonist potency to relative affinity to opiate receptors and the concentration of naloxone required for antagonism, for the prediction of the potential of new compounds to produce physical dependence.

## Introduction

In recent years, some benzomorphans related to cyclazocine have been shown to have unusual pharmacological properties. Two of these compounds are N-cyclopropylmethyl-5,9-dialkyl-2′-hydroxy-6,7-benzomorphans in which an oxo-group has been introduced at $C_8$ (Albertson, 1974). The other two compounds are N-dimethylfuryl-5,9-dimethyl-2′-hydroxy-6,7-benzomorphans (Merz, Langbein, Stockhaus, Walther & Wick, 1974). These compounds differ from other benzomorphans in that they have potent antinociceptive effects in the hot-plate test in mice but, rather unexpectedly, do not suppress the signs of morphine withdrawal in morphine-dependent monkeys (Villarreal & Seevers, 1972; Swain & Seevers, 1974). In tests on rodents, the compounds have either very little or no antagonist action (Archer, S. & Pierson, A.K., personal communication, 1972; Merz *et al.*, 1974) and in the morphine-dependent monkey, they do not cause a withdrawal syndrome (Villarreal & Seevers, 1972; Swain & Seevers, 1974).

In this paper, it will be shown that the normally close correlation between the relative agonist potencies found in the two preparations of the guinea-pig ileum and mouse vas deferens (Hughes,

Kosterlitz & Leslie, 1975b) does not hold for these compounds. Furthermore, it has been found that higher concentrations of naloxone are required to antagonize the agonist effects of these compounds than those of morphine, codeine, levorphanol and other congeners of morphine. Some of the results have been presented to a Work Session of the M.I.T. Neurosciences Research Program, May 1974 (Kosterlitz, 1975).

## Methods

### *Experimental procedures*

The methods used are those described earlier (Kosterlitz & Watt, 1968; Hughes *et al.*, 1975b). In principle, segments of ileum were stimulated coaxially (0.1 Hz, 0.5 ms duration, supramaximal voltage) and the mouse vas deferens by field stimulation (0.1 Hz, 1 ms, supramaximal voltage). For the determination of the relative agonist potency, dose-response curves were constructed; the concentration which caused a depression of 50% of the electrically evoked contraction was compared to a concentration of normorphine which gave the same depression. Normorphine was chosen as standard of reference because the onset and offset of action was more rapid than that of morphine, with which normorphine is equiactive in both preparations. Relative agonist potencies were calculated for each assay as the ratio $ID_{50}$ of normorphine/$ID_{50}$ of test substance.

The antagonist potency of naloxone was obtained by constructing dose-response curves of the benzomorphans in the absence and in the presence of 3 or 4 concentrations of naloxone. From the dose-ratios obtained, regression lines were calculated for log (dose-ratio $-1$) on log naloxone concentration. With all but one of the compounds, the slope of the regression line did not deviate significantly from unity; the equilibrium dissociation constant ($K_e$) was calculated from the intersection of the regression lines with the abscissa scale and expressed in nM. $K_e$ indicates the concentration of naloxone which causes a dose-ratio of 2.

The procedure for measurement of receptor affinity is based on the ability of a compound to prevent specific binding of [$^3$H]-dihydromorphine (Terenius, 1974a). A fraction enriched in synaptic plasma membranes was obtained from rat brain from which the cerebellum had been removed. The standard incubation mixture contained 0.8 nM [$^3$H]-dihydromorphine and a receptor fraction (0.4 mg of protein) in 0.4 ml of a solution of the following composition (mM): NaCl 124, KCl 5, $KH_2PO_4$ 1.2, $CaCl_2$ 0.75, $MgSO_4$ 1.3, N-2-hydroxy-ethylpiperazine-$N'$-2-ethanesulphonic acid (HEPES) 26; the pH was adjusted to 7.4 with 1M NaOH. Incubations, which were at 25°C for 40 min, were terminated by centrifugation. The radioactivity of the resulting pellets was measured by liquid scintillation spectrometry. In all experimental runs, samples containing an excess of unlabelled dihydromorphine (1 $\mu$M) were included; residual binding in these samples was considered to be nonspecific and subtracted from the experimental values (Goldstein, Lowney & Pal, 1971; Terenius, 1973; Pert & Snyder, 1973). The inhibitory effects of different concentrations of the test substances were expressed in % of the value obtained for specific binding of [$^3$H]-dihydromorphine. Each substance was tested at 4 or 5 concentrations in triplicate; values at each point did not alter normally by more than 10%. Each dose-response curve was repeated at least once.

### *Drugs*

The drugs used were as follows: normorphine hydrochloride, (−)-phenazocine hydrobromide, NIH 8152 as the hydrochloride (Dr E.L. May), ketocyclazocine and ethylketocyclazocine as free bases (Sterling-Winthrop; Dr S. Archer); Mr 1268 and Mr 1353 as the methanesulphonates and Mr 2034 as free base (C.H. Boehringer Sohn, Ingelheim; Dr H. Merz). The structures are given in Table 1. Stock solutions of the salts were made in distilled water, those of the bases after the addition of the required amount of HCl. Concentrations are given in nM.

[$^3$H]-dihydromorphine (52 Ci/mmol) was obtained from the New England Nuclear Corporation (Boston, Mass.).

## Results

### *Relative agonist potencies*

The benzomorphans which were assayed had no antagonist activity in rodent antinociceptive tests (Archer & Pierson, personal communication, 1972; Merz *et al.*, 1974; Merz, Stockhaus & Wick, personal communication, 1975) or in the guinea-pig ileum and mouse vas deferens (Kosterlitz & Waterfield, 1975; Kosterlitz, Waterfield & Leslie, 1975; unpublished observations). The dose-response curves for their agonist actions were parallel to that of normorphine or morphine in both *in vitro* preparations.

There were four compounds which had no physical dependence capacity in the morphine-dependent monkey, and one compound which had not been tested on the monkey (Table 1). Two of

**Table 1** Relative agonist potencies of benzomorphans in the guinea-pig ileum and the mouse vas deferens

| *Compound* | *Structure* | *Physical dependence capacity** | *Relative agonist potency (normorphine = 1)* | | *Mvd/Gpi* |
|---|---|---|---|---|---|
| | | | *Guinea-pig ileum* | *Mouse vas deferens* | |
| Normorphine | – | high | 1 | 1 | 1 |
| Phenazocine (NIH 7613) | (–)-α-5,9-dimethyl-2-phenethyl-2′-hydroxy-6,7-benzomorphan | high | 13.3 ± 1.1 (4) | 19.0 ± 3.6 (3) | 1.4 |
| NIH 8152 | (±)-β-2,9-dimethyl-5-*n*-propyl-2′-hydroxy-6,7-benzomorphan | high | 2.60 ± 0.29 (4) | 4.17 ± 0.52 (4) | 1.6 |
| Ketocyclazocine (NIH 8847) | (–)-α-5,9-dimethyl-8-oxo-2-cyclopropylmethyl-2′-hydroxy-6,7-benzomorphan | none | 68.5 ± 4.3 (9) | 16.3 ± 1.1 (6) | 0.24 |
| Ethylketocyclazocine (NIH 8848) | (–)-α-9-methyl-5-ethyl-8-oxo-2-cyclopropylmethyl-2′-hydroxy-6,7-benzomorphan | none | 397 ± 43 (7) | 99 ± 11 (7) | 0.25 |
| Mr 1268 (NIH 8735) | (±)-α-5,9-dimethyl-2-(2-methyl-3-methylfuryl)-2′-hydroxy-6,7-benzomorphan | none | 1.49 ± 0.15 (6) | 0.34 ± 0.03 (6) | 0.23 |
| Mr 1353 (NIH 8737) | (±)-α-5,9-dimethyl-2-(3-methylfurfuryl–2′-hydroxy-6,7-benzomorphan | none | 5.55 ± 0.50 (6) | 1.54 ± 0.10 (6) | 0.28 |
| Mr 2034 | (–)-α-(1R,5R,9R)-5,9-dimethyl-2-(L-tetrahydrofurfuryl)-2′-hydroxy-6,7-benzomorphan | ? | 94.4 ± 11.3 (5) | 22.0 ± 1.9 (4) | 0.23 |

The values are the means ± s.e. mean; the number of observations are given in parentheses. * Data obtained from Eddy & May (1966); Deneau, Villarreal & Seevers (1966); Villarreal & Seevers (1972); Swain & Seevers (1974).

them were ketocyclazocines, two were N-dimethylfuryl analogues of cyclazocine and one an N-tetrahydrofurfuryl analogue. Racemates were used or the (−)-isomers; the (+)-isomers were inactive (Kosterlitz, Waterfield & Berthoud, 1973; Kosterlitz *et al.*, 1975). Their relative agonist potencies, referred to normorphine as standard, varied between 1.5 and 400 in the guinea-pig ileum and 0.3 and 100 in the mouse vas deferens. The ratio, potency in mouse vas deferens/potency in guinea-pig ileum, varied only little around 0.25.

In contrast, the two benzomorphans with high physical dependence capacity, (−)-phenazocine and NIH 8152, had similar relative agonist potencies in the two preparations, the mouse vas deferens being somewhat more sensitive than the guinea-pig ileum. This good correlation is in agreement with the findings obtained on all other agonist compounds with either no or only negligible antagonist activity (Hughes *et al.*, 1975b).

*Sensitivity to the antagonist effect of naloxone*

The sensitivity of agonists to the antagonist action of naloxone is measured by the equilibrium dissociation constant, $K_e$. The values for seven agonists with relative potencies from 0.007 (codeine) to 690 (etorphine) varied between 1.2 and 4.0 nM, giving a mean of 2.2 nM (Kosterlitz, Lord & Watt, 1972).

**Table 2** Potency of naloxone to antagonize the agonist effects of normorphine and the benzomorphans listed in Table 1

| | *Guinea-pig ileum* | | *Mouse vas deferens* | |
|---|---|---|---|---|
| *Compound* | *Slope* | $K_e$ *(nM)* | *Slope* | $K_e$ *(nM)* |
| Normorphine | 0.95 ± 0.03 | 1.89 ± 0.15 (12) | 0.98 ± 0.05 | 3.10 ± 0.25 (6) |
| Phenazocine | 0.93 ± 0.06 | 2.08 ± 0.39 (5) | 1.06 ± 0.01 | 4.05 ± 0.19 (3) |
| NIH 8152 | 0.84 ± 0.05 | 1.26 ± 0.31 (4) | – | – |
| Ketocyclazocine | 1.04 ± 0.07 | 15.2 ± 3.1 (3) | – | – |
| Ethylketocyclazocine | 1.04 ± 0.05 | 14.9 ± 0.9 (4) | 0.98 ± 0.08 | 11.0 ± 0.6 (3) |
| Mr 1268 | 0.67 ± 0.02 | – | – | – |
| Mr 1353 | 0.91 ± 0.06 | 11.7 ± 1.0 (4) | – | – |
| Mr 2034 | 1.08 ± 0.02 | 10.4 ± 1.8 (7) | 1.00 ± 0.04 | 9.07 ± 0.71 (3) |

The values are the means ± s.e. mean; the number of observations are given in parentheses. The values of $K_e$, the equilibrium dissociation constant, were calculated from the intersection with the abscissa of the regression line obtained by plotting log (dose-ratio −1) against log[naloxone]. When the slope had a value of less than 0.8, the antagonism was considered to be non-competitive (Mr 1268) and no $K_e$ could be given. NIH 8152, the ketocyclazocines, Mr 1268 and 1353 were racemates, the remaining compounds the (−)-isomers.

**Table 3** Correlation between affinity to the opiate receptors in rat brain and agonist activity in the guinea-pig ileum and mouse vas deferens

| *Compound* | *Inhibition of [$^3$H]-dihydromorphine binding* $ED_{50}$ *(nM)* | *Relative affinity (normorphine = 1)* | *Relative agonist potency/ relative affinity (normorphine = 1)* | |
|---|---|---|---|---|
| | | | *Guinea-pig ileum* | *Mouse vas deferens* |
| Normorphine | 15 | 1.0 | 1.0 | 1.0 |
| (−)-Phenazocine | 0.8 | 19 | 0.7 | 1.0 |
| (−)-Ketocyclazocine | 2.0 | 7.5 | 9.1 | 2.2 |
| (−)-Ethylketocyclazocine | 0.35 | 43 | 9.2 | 2.3 |
| Mr 1268 | 17 | 0.88 | 1.7 | 0.39 |
| Mr 1353 | 10 | 1.5 | 3.7 | 1.0 |
| Mr 2034 | 0.4 | 38 | 2.5 | 0.6 |

The $K_e$ of naloxone determined for normorphine, (−)-phenazocine and NIH 8152 as agonists varied in the guinea-pig ileum between 1.3 and 2.1 nM (Table 2). In the less robust mouse vas deferens, such estimations were more difficult because of the long duration of the assay; the values for normorphine and phenazocine varied between 3 and 4 nM.

When the $K_e$ values of naloxone were determined for the benzomorphans with no physical dependence capacity, they varied between 10 and 15 nM in the guinea-pig ileum and 9 and 11 nM in the mouse vas deferens. These results mean that, in the guinea-pig ileum, these benzomorphans require 6 to 7 times more naloxone for antagonism than normorphine. On the other hand, the two benzomorphans with high physical dependence capacity were as sensitive to the action of naloxone as was normorphine. Although there are fewer data for the mouse vas deferens, the relationship is similar, the benzomorphans with no physical dependence capacity requiring about 3 times more naloxone than normorphine.

*Correlation between agonist potency and inhibition of specific [$^3$H]-dihydromorphine binding*

The benzomorphans show specific affinities to the opiate receptors as demonstrated by their ability to inhibit specific binding of [$^3$H]-dihydromorphine to membrane fragments of rat brain (Table 3). The slopes of the dose-inhibition curves were not different from those obtained for morphine or other agonists (Terenius, 1974b). The rank order of their relative affinities is highly correlated to that of their relative agonist potencies in both *in vitro* preparations. When the ratios of relative agonist potency to relative affinity were calculated, their values were closer to the expected value of unity in the mouse vas deferens than in the guinea-pig ileum (Table 3).

## Discussion

In their report on the effects of the N-cyclopropylmethyl - 5,9 - dialkyl - 2′ - hydroxy - 8 - oxo 6,7-benzomorphans, Swain & Seevers (1974) stressed the very unusual nature of these compounds. They neither suppressed nor precipitated abstinence in morphine-dependent monkeys. Similar findings had been obtained by Villarreal & Seevers (1972) on the N-dimethylfuryl-5,9-dimethyl-2′-hydroxy-6,7-benzomorphans. The importance of these findings lies in the fact that these substances are compounds which have potent antinociceptive effects without antagonist activity and yet have no physical dependence capacity in monkeys.

The results obtained in the guinea-pig ileum and mouse vas deferens underline their unusual pharmacological properties. The low sensitivity of the mouse vas deferens to the agonist action of these drugs and the lowered effectiveness of naloxone as an antagonist indicate that the mode of action of these drugs is probably different from that of agonists of the morphine type. It has been shown previously (Kosterlitz *et al.*, 1972) that the antagonist potency of naloxone is lower against the agonist actions of compounds with dual agonist and antagonist effects, such as nalorphine, levallorphan and diprenorphine, than against morphine. The benzomorphans investigated now, are the first compounds without antagonist component to show this phenomenon. Notwithstanding the close rank order correlation between the affinity of these compounds to the opiate receptors and their agonist potencies in both *in vitro* preparations, it has to be considered that they may act on a receptor different from that which mediates the effects of the agonists of the morphine type. In this connection it is of interest to note that the endogenous ligand enkephalin, which is an agonist without antagonist activity, also requires more naloxone for its antagonism (Hughes, Smith, Morgan & Fothergill, 1975a; Kosterlitz & Hughes, 1975).

The three assays described may thus be suitable for the selection of antinociceptive compounds that will not produce physical dependence, always providing that freedom from this property in monkeys also holds for man.

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