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Use of the mouse jumping test for estimating antagonistic potencies of morphine antagonists

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The potencies of 19 reference morphine antagonists have been compared in a modified version of the mouse jumping test. Mice were each implanted subcutaneously with one 75 mg pellet of morphine. Antagonist challenge took place 72 h later and the incidence of repetitive vertical-jumping was monitored over 1 h. A high Pearson correlation coefficient ($r = 0{\cdot}997$) was found between quantitative assays based on the total number of jumps per mouse and quantal assays based on mice jumping at least 6 times. A comparison of relative potencies obtained with the mouse test and with non-withdrawn morphine-dependent monkeys gave a Spearman rank order coefficient of 0·91 while a similar comparison with values obtained with the guinea-pig isolated ileum preparation also gave a high correlation coefficient ($r = 0{\cdot}92$). Whereas it is difficult to assess the antagonistic component of buprenorphine and cyclorphan with the ileum preparation, both compounds can be satisfactorily assayed in the mouse jumping test. The reported antagonistic properties of ketocyclazocine and profadol could not be confirmed in the mouse model.

In the pharmacological evaluation of novel analgesics, the ability of a new compound to prevent or antagonize the actions of opioids has often been considered a useful, but by no means certain, indication of low morphine-like physical dependence capacity. Recently, the ratio of the antagonist and agonist potencies of an analgesic, rather than the individual components, has been emphasized in the search for efficacious alternatives to morphine and pentazocine (e.g. Kosterlitz, Waterfield & Berthoud, 1974). These ratios may be obtained using the guinea-pig isolated ileum (Kosterlitz & Watt, 1968) or mouse isolated vas deferens (Hughes, Kosterlitz & Leslie, 1975) preparations or a combination of several well-known antinociceptive tests in rodents, e.g. mouse writhing (for agonism) with either mouse tail flick or rat tail pressure tests (for morphine antagonism). Alternatively, the agonist-antagonist nature of analgesics may be compared by measuring their abilities to block [^{3}H]naloxone binding to "opiate receptors" *in vitro* in both the presence and absence of sodium ions (Pert & Snyder, 1974).

To fully characterize the antagonist component of analgesics, work in this laboratory has been directed towards the investigation of additional *in vitro* and *in vivo* methods. This has proved necessary since, at one extreme, the antagonistic activity may be over-estimated with some compounds in the tail flick test when warm water is used as the nociceptive stimulus (for example, levorphanol has a morphine antagonist potency of the same order as cyclazocine in this procedure) while at the other extreme, several oripavine narcotic antagonist analgesics, despite precipitating abstinence in non-withdrawn morphine-dependent monkeys, are unable to antagonize the antinociceptive effect of morphine in the tail pressure test (Cowan, Lewis & Macfarlane, unpublished work).

In the present paper, the antagonistic potencies of standard narcotic antagonists have been assessed using a modified version of the mouse jumping test (Saelens, Granat & Sawyer, 1971) and the values compared with those described in the literature from studies using the guinea-pig isolated ileum and the morphine-dependent monkey.

MATERIALS AND METHODS

Four groups of 15 male mice (MFI/O1a, 22–25 g) were lightly anaesthetized with halothane and implanted in the dorsal subcutaneous tissue with a specially formulated morphine pellet (75 mg morphine base, 2 mg polyvinylpyrrolidone and 1 mg magnesium stearate). In contrast to the formulation of Gibson & Tingstad (1970), these pellets contained a minimal amount of excipient and the resulting smaller size was considered more convenient for dependence studies with mice.

Approximately 72 h after implantation, 12 unfasted mice from each group were individually housed in the monitoring apparatus previously described (Cowan & Cowan, 1972) and those animals exhibiting more than 5 exploratory jumps over the following 0·5 h were rejected. Jumping occurred infrequently at this time and only 0·60% of the mice used had to be discarded.

Mice from each group were injected with one of four different doses of morphine antagonist and at a constant volume of 10 ml kg^{-1}. The number of vertical jumps occurring during successive 15 min periods was recorded automatically. The number of mice jumping at least 6 times during the 1 h session was expressed as a percentage of the total in the group and the median antagonistic dose (AD 50) and 95% confidence limits for each antagonist were computed using a logit analysis (Finney, 1971). Parallelism of the dose-response lines was tested by the chi-squared test, and potency ratios with 95% confidence limits were computed (using Bliss 17, a program written by Prof. D. J. Finney). Potency ratios were obtained from quantitative data (transformed to a logarithmic scale) by using a parallel line assay program.

Compounds used

Buprenorphine hydrochloride (Reckitt & Colman), butorphanol tartrate (Bristol), cyclazocine (Sterling-Winthrop), cyclorphan hydrochloride (gift from Dr. W. Leimgruber, Roche), cyprenorphine and diprenorphine hydrochlorides (Reckitt & Colman), etazocine hydrochloride (α-(−)-2′-hydroxy-2-methyl-5,9-diethyl-6,7-benzomorphan, gift from Dr. E. L. May, NIH, USA), GPA 1657 (β-(−)-2′-hydroxy-2,9-dimethyl-5-phenyl-6,7-benzomorphan, Geigy), ketocyclazocine (α-2-cyclopropylmethyl-8-keto-5,9-dimethyl-2′-hydroxy-6,7-benzomorphan, Sterling-Winthrop), levallorphan and levorphanol tartrates (Roche), metazocine hydrobromide (α-(−)-2,5,9-trimethyl-2′-hydroxy-6,7-benzomorphan, gift from Dr. W. R. Martin, NIDA, USA), nalbuphine hydrochloride (Endo), nalorphine hydrobromide, B.P. (Burroughs Wellcome), naloxone and naltrexone hydrochlorides (Endo), pentazocine lactate (Sterling-Winthrop), profadol hydrochloride (Parke, Davis & Co.), propiram fumarate (Bayer) and viminol-*p*-hydroxybenzoate (S_2-stereoisomer, Zambon).

Cyclazocine and ketocyclazocine were each dissolved in a minimal amount of 0·1 N HCl, the pH adjusted to 5·0 with $NaHCO_3$ solution and made up to volume with distilled water. Other compounds were dissolved or diluted in physiological saline solution and all were administered subcutaneously except for GPA 1657 and viminol which were suspended in 0·1% Tween 80 and given intraperitoneally. All doses are expressed in terms of the free base.

RESULTS

The potencies of several reference narcotic antagonists (relative to nalorphine) in the mouse jumping test are presented in Table 1. Although these values are based on either quantal or quantitative criteria, the Pearson correlation coefficient between the two sets of results is very high ($r = 0.997$, $P < 0.001$). With the exception of buprenorphine, jumping occurred most frequently between 0–15 min after injection of each dose of active compound and was essentially over after a further 15 min (Fig. 1).

When the (quantal) potency ratios are compared with those obtained at the University of Michigan from non-withdrawn morphine-dependent rhesus monkeys (Table 1), the Spearman rank order coefficient is 0·91. There is also a good agreement ($r = 0.92$, $P < 0.01$) between the relative potencies of those antagonists that can be assayed in both the mouse jumping test and the guinea-pig isolated ileum procedure (Kosterlitz

Table 1. *Potencies of morphine antagonists, relative to nalorphine, in morphine-dependent mice and monkeys.*

Compound	Mouse (quantitative assay)	Mouse (quantal assay)			Monkey
	Potency ratio (95% limits)	AD50, mg kg^{-1} (95% limits)	Slope (± s.e.)	Potency ratio (95% limits)	Potency ratio
Naltrexone	43·2 (30·4–61·4)	0·012 (0·009–0·016)	1·59 (0·47)	46·9 (29·5–74·1)	6–13 (6)
Diprenorphine	32·0 (17·8–57·4)	0·022 (0·013–0·031)	1·23 (0·33)	26·1 (15·8–49·2)	16 (6)
Cyprenorphine	25·6 (14·7–44·4)	0·025 (0·013–0·047)	0·86 (0·28)	22·6 (11·2–48·6)	15–20 (6)
Naloxone	23·5 (13·6–40·6)	0·027 (0·015–0·056)	0·89 (0·37)	21·2 (11·1–40·9)	7 (6)
Levallorphan	7·6 (3·5–16·5)	0·093 (0·044–0·17)	0·69 (0·19)	6·2 (2·9–14·8)	4 (6)
Cyclorphan	5·8 (2·8–11·7)	0·10 (0·060–0·21)	0·72 (0·22)	5·8 (2·7–11·7)	3·5 (5)
Cyclazocine	6·7 (4·0–11·1)	0·13 (0·072–0·21)	0·97 (0·26)	4·6 (2·4–9·1)	3·5 (1)
Buprenorphine	1·2 (0·53–2·6)	0·40 (0·25–1·5)	0·94 (0·40)	1·4 (0·65–2·7)	
Nalorphine	1·0	0·57 (0·32–1·3)	0·92 (0·39)	1·0	1·0
Butorphanol	0·79 (0·43–1·4)	0·72 (0·47–1·2)	0·97 (0·28)	0·79 (0·43–1·4)	
Nalbuphine	0·25 (0·16–0·40)	2·6 (0·35–4·2)	1·29 (0·65)	0·22 (0·13–0·48)	0·01 (6)
Pentazocine	0·083 0·040–0·18)	10·1 (5·8–19·2)	0·79 (0·22)	0·057 (0·027–0·12)	0·02 (3)
Ketocyclazocine		>10			
GPA 1657		>30			0·05 (4)
Etazocine		>30			0·05 (2)
Metazocine		>30			0·03 (4)
Profadol		>50			0·01 (6)
Viminol		>50			
Propiram		>100			

Sources of values: (1) Deneau & Seevers (1962); (2) Deneau, Villarreal & Seevers (1966); (3) cited in Jacobson (1972); (4) Villarreal (1970); (5) Villarreal (1972); (6) Villarreal & Karbowski (1974).

The quantitative assays are based on the number of jumps recorded with 4 groups of 12 mice during 1 h. Quantal data are obtained from the same experiments; mice jumping at least 6 times during 1 h are considered to show a positive response.

& others, 1974). The correlation between the 9 compounds that have been compared is highlighted in Fig. 2.

Median antagonistic doses could not be estimated for GPA 1657, etazocine, metazocine, viminol and propiram since only 1–2 mice jumped in each instance. The highest dose of these compounds was limited to the range 30–100 mg kg^{-1} by solubility problems and/or the appearance of side-effects in the animals. No jumping was recorded during the 1 h experimental session with mice given either saline, ketocyclazocine (0·3, 1, 3 and 10 mg kg^{-1}), levorphanol (0·3, 1, 3 and 10 mg kg^{-1}) or profadol (1, 10, 30 and 50 mg kg^{-1}). Other signs of abstinence such as diarrhoea or paw tremor were not observed with these compounds.

DISCUSSION

Since the original observations of Huidobro and Way and their respective colleagues (e.g. Maggiolo & Huidobro, 1961; Way, Loh & Shen, 1969), repetitive vertical-jumping has become the most frequently measured sign of (morphine) abstinence in the mouse. Thus, more recently, the incidence of jumping has been claimed to provide an objective index of the physical dependence capacity of reference, and perhaps new, analgesics (Marshall & Weinstock, 1971; Saelens & others, 1971). These studies in mice have been modelled on procedures that are well-established with monkeys, involving (i), direct dependence tests followed by naloxone challenge (Lauldi & Carenzi, 1974), (ii), single-dose suppression tests (Takemori, Stesin & Tulunay, 1974) and (iii), attempts to block the naloxone-induced abstinence syndrome (Iorio, Deacon & Ryan, 1975). In these experiments, naloxone has been used almost exclusively to precipitate the abstinence syndrome. Since available information on the potencies of different antagonists in the jumping test is limited to a very few reports (e.g. Giering, Davidson & others, 1974), the major aim of the present work has been

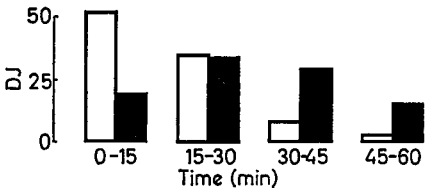

FIG. 1

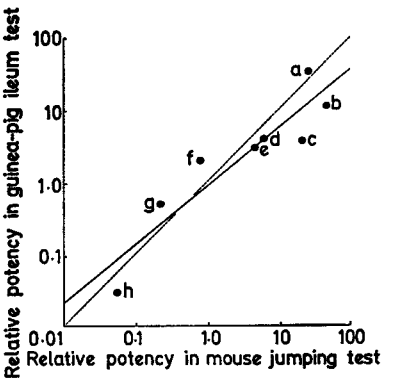

FIG. 2

FIG. 1. Incidence of antagonist-induced jumping shown by groups of 12 morphine-pelleted mice at 15 min intervals over 1 h. The histograms are based on the jumping data used in calculating the relative potencies of buprenorphine (solid columns) and the remaining compounds listed under *Materials and Methods* (open columns). DJ—percentage distribution of jumping.

FIG. 2. Correlation between the relative antagonistic potencies of narcotic antagonists (nalorphine = 1) obtained with morphine-pelleted mice (quantal assay) and with the guinea-pig isolated ileum preparation (Kosterlitz & others, 1974 and H. W. Kosterlitz, personal communication). The values are plotted on a logarithmic scale. Correlation coefficient, r = 0·92. The solid line has been drawn from $\log_{10} y = 0{\cdot}807 \log_{10} x - 0{\cdot}049$ and its slope does not differ significantly from unity (dotted line). a—diprenorphine, b—naltrexone, c—naloxone, d—levallorphan, e—cyclazocine, f—butorphanol, g—nalbuphine, h—pentazocine.

to compare a wide variety of narcotic antagonists in this test situation under strictly standardized conditions.

A good overall relation exists between the potencies (relative to nalorphine) of standard antagonists when they are evaluated in either the mouse jumping test or in the guinea-pig isolated ileum procedure (Fig. 2). Similarly, when potencies obtained with either morphine-dependent mice or morphine-dependent monkeys are compared, a high Spearman rank order coefficient is obtained. In comparison with the other two tests, the mouse model is particularly sensitive to the noroxymorphone derivatives, naloxone and naltrexone. At the other extreme, potency ratios could not be obtained with GPA 1657, etazocine, metazocine, profadol and propiram in the present study whereas very low ratios (0·01–0·05) have been reported for the first four compounds in the monkey (references at the foot of Table 1). However, the infrequent jumping that *did* occur after injection of GPA 1657, etazocine, metazocine and propiram distinguishes these compounds from profadol and saline and permits them to be classified as "very weak morphine antagonists" at least in the mouse. In this context it should be stressed that both etazocine and propiram give flat dose-response lines in non-withdrawn morphine-dependent monkeys (Deneau, Villarreal & Seevers, 1966; Villarreal & Seevers, 1967) while metazocine is not an antagonist in the guinea-pig isolated ileum (H.W. Kosterlitz, personal communication). In contrast to GPA 1657, profadol precipitates signs of abstinence in men dependent on 240 mg of morphine per day (Jasinski, Martin & Hoeldtke, 1971). Whereas the clinical finding with GPA 1657 is consistent with the very low incidence of jumping elicited by this benzomorphan in mice, the differing results obtained with profadol may indicate a serious weakness in the rodent model.

The proposition that the antinociceptive agent, ketocyclazocine, possesses antagonistic properties is based on its weak antagonism of morphine and lack of agonist effect in the rat tail flick (radiant heat) test (Michne, Pierson & Albertson, 1974). Despite this profile, ketocyclazocine does not precipitate abstinence in non-withdrawn morphine-dependent monkeys (Swain & Seevers, 1974) and is not an antagonist in the isolated ileum preparation (Kosterlitz & others, 1974). The inability of ketocyclazocine (0·30–10 mg kg^{-1}, s.c.) to induce jumping in morphine-pelleted mice is in accord with the monkey and *in vitro* studies.

A very low incidence of jumping was recorded in mice after injecting the S_2-stereoisomer of the *N*-benzylpyrrylethanolamine derivative, viminol. This was a surprising observation since the S_2-compound has previously been reported to elicit appreciable jumping in mice receiving multiple injections of morphine over two days (Della Bella, Ferrari & others, 1973). A possible explanation could be that the S_2-stereoisomer induces jumping in only certain strains of mice (D. Della Bella, personal communication). When antagonists of low activity, such as viminol, are being evaluated it would seem beneficial to check for the presence of other abstinence signs (e.g. diarrhoea). Using the apparatus described in the present work, it is a simple matter to routinely assess the amount of faeces that has collected over 1 h when the mice are finally removed from their individual bottles. Excessive defaecation was not observed at the end of those experiments involving viminol S_2-stereoisomer, this finding being in keeping with the corresponding jumping data.

Two types of compound are particularly difficult to evaluate on the guinea-pig isolated ileum: (i), antagonists with marked agonist activity e.g. cyclorphan (which has recently been characterized on the mouse isolated vas deferens by Hughes & others,

1975) and (ii), antagonists with long half-times for onset and offset of the agonist effect, e.g. buprenorphine (Kosterlitz, Leslie & Waterfield, 1975). Both cyclorphan and buprenorphine can be satisfactorily assayed using the version of the mouse jumping test described in the present paper.

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