COMMUNICATIONS

Reversal of yeast-induced motor impairment in rats as a test for narcotic and non-narcotic analgesics

Some of the tests currently used to assess antinociceptive activity in rodents either lack the necessary specificity (Hendershot & Forsaith, 1959) or fail to detect potentially useful analgesic agents (D'Amour & Smith, 1941). Other methods involve the use of cumbersome (Green, Young & Godfrey, 1951) or expensive (Perrine, Atwell & others, 1972) equipment and the practice of confining animals in unfamiliar environments (Grotto, Dikstein & Sulman, 1965; Perrine & others, 1972).

We describe here a modification of the well known test of Randall & Selitto (1957) which may be less stressful for the rats, requires no expensive or specialized apparatus and detects clinically useful narcotic, narcotic antagonist and antipyretic analgesics.

Male albino Sprague-Dawley rats, 140–180 g, were deprived of food overnight and throughout experiments when drugs were given by mouth. In other tests, the animals had free access to food and water. Rats were injected in the left hind paw with 0·2 ml of a 40 % w/v suspension of Brewers' yeast (Distillers) in physiological saline. After a a specified time, groups of 10 rats were dosed randomly with the test drugs. Five h after the yeast injection, each rat was allowed to walk across and explore the metal grid of its closed cage top. At this time an assessment of the gait of the animals was made by an observer who was unaware of the dosage schedule (scoring system: 0 = three-legged gait; 0·5 = marked limping; and 1 = normal gait, i.e. antinociception). The number of rats with a score of 1 was expressed as a percentage of the total number of rats in each group. Two animals with a score of 0·5 were counted as one animal exhibiting analgesia. The dose of each drug required to produce an antinociceptive response in 50 % of the rats was calculated using the minimum logit chi-squared method correcting for natural responses (Berkson, 1953).

Compounds used. Acetic acid (Hopkin & Williams); acetylsalicylic acid; (+)-amphetamine sulphate (Sigma); buprenorphine hydrochloride (RX 6029–M) (Reckitt and Colman); chlorpheniramine maleate (Allen & Hanburys); chlorpromazine hydrochloride (May & Baker); codeine phosphate; cyclazocine (Winthrop); hydroxyzine dihydrochloride (Pfizer); ibuprofen (Boots); indomethacin (Merck Sharp & Dohme); morphine sulphate (Macfarlan Smith); paracetamol; pentazocine lactate (Winthrop); pethidine hydrochloride (Macfarlane Smith); phenylbutazone (Chelsea Drug); profadol hydrochloride (Parke Davis) and propranolol hydrochloride (ICI).

The antinociceptive activities of three classes of analgesic are presented in Table 1 along with a selection of compounds that are not used clinically as analgesics. Shallow dose-response curves were often obtained, particularly with the weak analgesics, and this is reflected in the wide 95 % confidence limits of the calculated ED50 values. Nevertheless, the results show that the present simplification of the inflamed paw test of Randall & Selitto (1957) is capable of detecting activity with representatives of each of the 3 main analgesic classes. Furthermore, the proposed method may be less aversive for the animals since it merely involves the natural function of walking in a familiar environment.

The scoring system used is subjective and perhaps may be considered lacking in precision. These points should be balanced against the merits of the test. Thus, the sensitivity towards antipyretic analgesics is higher than in other commonly used tests (Hendershot & Forsaith, 1959; Paalzow, 1969); the antinociceptive activities

Table 1. *Antinociceptive activities of several classes of pharmacological agents on yeast-induced motor impairment in rats.*

Classification	Drug	Route*	ED50,† mg kg^{-1} (95% confidence limits)
Antipyretic-analgesics	Indomethacin	} oral	1·21 (0·41–3·53)
	Ibuprofen		5·53 (0·32–95)
	Phenylbutazone		12 (5·63–27)
	Acetylsalicylic acid		78 (31–200)
	Paracetamol		146 (39–543)
Narcotic analgesics	Morphine	} s.c.	1·93 (0·45–8·34)
	Codeine		7·05 (1·26–39)
	Pethidine		9·66 (1·07–88)
Narcotic antagonist analgesics	Buprenorphine	} s.c.	0·12 (0·05–0·32)
	Cyclazocine		0·93 (0·17–5·11)
	Profadol		3·63 (1·72–7·70)
	Pentazocine		6·79 (1·23–37)
Miscellaneous	Amphetamine	s.c.	0·90 (0·31–2·66)
	Acetic acid	i.p.	155 (31–788)
	Chlorpromazine	} s.c.	>3
	Propranolol		>10
	Hydroxyzine		>30
	Chlorpheniramine		>100

* For oral dosing, each drug was suspended in 5% w/v gum acacia and administered 3 h after the yeast injection. For parenteral dosing, each drug was either dissolved or diluted in physiological saline and administered either 4·5 h (s.c.) or 3 h (i.p.) after the yeast injection.

† Doses expressed in terms of the salt, where applicable, except for buprenorphine and pentazocine.

of narcotic antagonists can be satisfactorily assessed; a reasonable specificity can be claimed for the procedure; and the rank order of compounds in each class is similar to that found in man (Collier, Dinneen & others, 1968).

The observation that acetic acid, which has been shown to possess systemic anti-inflammatory properties (Garattini, Jori & others, 1965; Atkinson, 1971), also exerts a weak antinociceptive action is in keeping with the results of other workers (Winter & Flataker, 1965; Hitchens, Goldstein & others, 1967) who have used different irritants. The antinociceptive effects of (+)-amphetamine have also been reported (e.g. Shemano, Hitchens & others, 1968).

Langford, Holmes & Emele (1972) have recently described the effects of mild analgesics on the locomotor activity of mice injected with yeast in both hind feet. It is of interest that these workers failed to detect the antinociceptive activities of codeine and paracetamol whereas the antihistamine, chlorpheniramine, produced a highly significant effect. These findings contrast with the data in Table 1 and reinforce the suggestion that the present test may afford a greater degree of sensitivity and specificity in the primary screening of weak and strong analgesics.

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Department of Pharmacology,
Pharmaceutical Research Laboratories,
Reckitt and Colman Pharmaceutical Division,
Dansom Lane, Hull HU8 7DS, U.K.
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D. C. ATKINSON
A. COWAN

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Reversal by prostaglandin E_2 of the inhibitory effect of indomethacin on contractions of guinea-pig ileum induced by angiotensin

One of the putative roles for prostaglandins is in the modulation of neurohumoral transmission and hormone action (Hedqvist, 1970; Horton, 1969). It is possible that some of the multiplicity of angiotensin actions could be due to the interaction of prostaglandins and angiotensin. We have recently shown (Chong & Downing, 1973) that contractions of a variety of smooth muscle preparations induced by angiotensin II could be inhibited by indomethacin, which is a potent prostaglandin biosynthesis inhibitor (Vane, 1971). Indomethacin has also been shown to inhibit electrically-induced contractions of the guinea-pig ileum and the inhibition could be reversed by prostaglandins (Ehrenpreis, Greenberg & Belman, 1973). Further evidence is now presented for the involvement of prostaglandins in the contraction of the guinea-pig ileum by angiotensin II.

Segments of guinea-pig ileum 20–30 mm in length were suspended in aerated Tyrode solution in a 15 ml organ bath maintained at $34 \pm 1°$. Contractile responses were recorded by means of an isometric transducer. Prostaglandin E_2 (PGE$_2$) solutions (1 mg ml^{-1}) were prepared in 25% ethanol and kept frozen, dilutions were made in distilled water immediately before use. Indomethacin solution was prepared by dissolving it in a slight excess of sodium carbonate solution, making up to the desired volume with Tyrode solution and adjusting to pH 7·3, just before use.

Indomethacin ($5·6 \times 10^{-5}$M) caused $46·1 \pm 4·7\%$ and $52·8 \pm 6·4\%$ reductions of the fast and slow components respectively of the submaximal contractile response