J. Pharm. Pharmac., 1967, **19**, 367–373 Received January 6, 1967

Blockade of bradykinin-induced nociception in the rat as a test for analgesic drugs with particular reference to morphine antagonists

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A rat test, recently outlined by Deffenu & others for the evaluation of analgesic drugs and using intra-arterially administered bradykinin as the nociceptive stimulus, has been applied to varied categories of analgesic drugs including, for the first time, morphine antagonists. For purposes of comparison, data obtained with the same drugs using established techniques are also given. The use of the bradykinin-antagonism test as a laboratory model with which to investigate drugs of potential therapeutic value in man is discussed and compared particularly with the mouse phenylquinone-induced writhing test. Attention is drawn to the possibility of correlating the effect of drugs on bradykinin-induced pseudo-affective responses in animals with human pain.

NALORPHINE, pentazocine and related morphine antagonist drugs, which are inactive in established laboratory tests for antinociceptive activity like the rat tail-pressure test, are now known to be active in the mouse phenylquinone-induced writhing test (Taber, Greenhouse & Irwin, 1964; Blumberg, Wolf & Dayton, 1965; Pearl & Harris, 1966), the rat paw-oedema test (Winter & Flataker, 1965) and a modification of the Randall-Siletto rat tail test (Ward, Foxwell & Funderbunk, 1965).

The assessment of analgesic drugs by their ability to block the responses of animals to intra-arterial injections of chemical algesic agents was first discussed by Lim (1960), and was investigated in some depth by Guzman, Braun & Lim (1962) using lightly anaesthetized cats and dogs. Bradykinin, in a minimum effective dose of 1–2 μg, was by far the most potent algesic agent in provoking a "pseudo-affective" response characterized by a fleeting vocal response, hyperpnoea and hypertension. In 1964, Guzman, Braun, Lim & others evaluated some well-known drugs in the conscious dog, using as the criterion for analgesia the block of the vocal response evoked by injection of bradykinin into the splenic artery. Deffenu, Pegrassi & Lumachi (1966) recently adapted the Guzman technique to rats. Small doses of bradykinin injected retrogradely into the right carotid artery of conscious animals by way of a previously implanted catheter resulted in a syndrome consisting of dextrorotation of the head, flexion of the right fore-limb and, occasionally, squeaking. ED50 values were reported for methadone, phenylbutazone, codeine, aspirin and phenacetin. Aminopyrine was also active in this modified test but antagonist-analgesics such as nalorphine were not mentioned.

We report here the results we have obtained using the technique of Deffenu & others and compare them with data obtained using two conventional laboratory techniques for the evaluation of analgesic drugs, and also with the potency of these drugs in man.

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Methods

The technique used to cannulate the rats differed only in minor detail from that outlined by Deffenu & others (1966). Male S.P.F.-derived Sprague–Dawley rats of between 250 and 350 g were lightly anaesthetized with ether, and a polythene cannula (internal diameter 0·40 mm, Portland Plastic PP. 25) tied into the right carotid artery with the tip pointing towards the heart. A trochar was used to deliver the free end through the skin of the dorsal surface in the scapular region. Having established that the cannulation was successful, 0·2 ml of saline was injected into the tube and the open end closed with a tightly fitting polythene cap similar to the type used by Weeks & Jones (1960). The skin wound in the neck was closed by application of Michelle clips and the anaesthetic discontinued. Deffenu & others (1966) used their animals 1 hr after recovery from anaesthesia but we allowed at least 4 hr to elapse before testing and mostly tests were made on the day after cannulation.

The minimum dose of bradykinin required to consistently produce dextrorotation of the head and flexion of the right fore-limb was established for each rat. This dose was commonly 0·05 or 0·10 μg and rats not responding to 0·50 μg were discarded. Rats did not always squeak after these threshold doses of bradykinin and disappearance of the head rotation and fore-limb flexion were taken as the criteria for scoring an analgesic effect in animals after drug administration. Bradykinin was given as a solution of the pure synthetic peptide (Sandoz) in 0·2 ml of 0·9% saline and was washed in immediately with a further 0·2 ml of saline. The response developed within about 5 sec and persisted for about a further 10 sec. Compounds under test were administered by oral, subcutaneous or intraperitoneal route, after which the established threshold dose of bradykinin was injected at regular intervals until the response returned. In control animals there was no evidence that tachyphylaxis developed after repeated injections of bradykinin. For any given drug and dose level, the percentage of rats failing to respond to the algesic stimulus could be plotted against time to give a clear presentation of the rate of onset, duration, and decay of activity (Fig. 1).

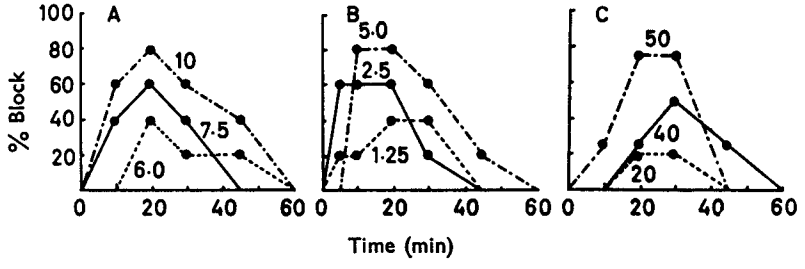

FIG. 1. Representative response curves obtained after the administration of analgesic drugs to rats receiving liminal intra-arterial doses of bradykinin at regular intervals. A. Fentanyl (μg/kg). B. M285 (mg/kg). C. Phenylbutazone (mg/kg). Each curve based on the mean response of 5 rats to a dose of analgesic administered subcutaneously at zero time. Ordinate shows percentile block of the bradykinin-induced nociceptive syndrome.

With all drugs the ED50 values at the time of peak effect and their 95% confidence limits were estimated using the method of Litchfield & Wilcoxon (1949).

Details of the rat tail-pressure test and the mouse anti-writhing test, against which the bradykinin antagonizing effects of drugs have been compared, have been published previously (Boura & Fitzgerald, 1966). The tail-pressure test was essentially similar to that described by Green & Young (1951), where animals were regarded as showing analgesia if they failed to squeal on application of a pressure greater than twice the mean pressure required to cause a vocal response in the control. Relative analgesic efficacy in mice was determined as the dose of drug required to reduce by 50% the number of abdominal stretches caused by the intraperitoneal injection of 2 ml/kg of phenyl-*p*-benzoquinone (Hendershot & Forsaith, 1959).

Narcotic antagonist activity was assessed as the dose of drug which would, in the rat tail-pressure test, reduce to 50% the total analgesia caused by 10 mg/kg morphine sulphate administered subcutaneously (Green, Ruffell & Walton, 1954).

All doses are expressed as the weight of the salt used, where applicable.

Results and discussion

To explore fully the potentialities and limitations of the bradykinin-antagonism technique, representatives from a variety of classes of analgesic drugs were examined in this and the other tests. The drugs and the results obtained are listed and classified in Table 1. The route of administration where possible was the same as that normally used in man. Aspirin, phenylbutazone and codeine, had been examined by Deffenu & others (1966) and our results are in fair agreement with theirs.

Results obtained in some typical bradykinin-antagonism tests are depicted graphically in Fig. 1, and the dose-response lines obtained with four drugs at the time of peak-effect are shown in Fig. 2. Although three different classes of analgesic are represented (strong narcotic—morphine, narcotic antagonist—nalorphine, and antipyretic/anti-inflammatory agents —aspirin and phenylbutazone) the lines do not differ significantly from parallelism at the 95% level of confidence.

The anti-inflammatory analgesics were inactive in the rat tail-pressure test (ED50 $\geqslant$ 100 mg/kg) but active in the Hendershot & Forsaith test with the relation between the ED50 values for aspirin and phenylbutazone, at least, being of the same order as that of the dose levels which are most commonly used in man. Aspirin and phenylbutazone were active in the anti-bradykinin test only when given intraperitoneally, and the relation between their ED50 values was almost identical to that seen in the Hendershot & Forsaith test. However, the doses required to block the bradykinin-induced syndrome in rats were some five times greater than those needed to antagonize phenylquinone-induced writhing in mice. Mefenamic acid was totally inactive at sub-toxic doses by any route in the bradykinin-antagonism test.

TABLE 1. ACTIVITY OF A VARIETY OF ANALGESIC DRUGS IN LABORATORY ANIMAL TESTS AND IN MAN

Class of analgesic	Representative drugs	Animal tests (ED50 mg/kg)				Approximate human analgesic dose
		Rat tail pressure	Mouse H & F	Anti-bradykinin rat	Morphine antagonism	
Anti-inflammatory weak analgesic with peripheral action	Aspirin	⩾100 oral ⩾100 s.c./i.p.	100·0 oral 22·0 s.c.	⩾500 oral 125 i.p.	—	300–600 mg oral
	Phenylbutazone	⩾100 i.p.	25·0 i.p. 5·6 s.c. 12·0 i.p.	32·0 i.p.	—	100–200 mg oral
	Mefenamic acid	⩾100 i.p.	43·0 i.p.	>300 i.p.	—	500 mg oral
Weak analgesic central action	Codeine phosphate	17·0 s.c.	5·6 s.c.	38·5 s.c.	—	60 mg oral/s.c.
Strong analgesic central action	Morphine hydrochloride	2·5 s.c.	0·64 s.c.	1·1 s.c.	—	10–15 mg. s.c./i.m.
	Etorphine hydrochloride (M99)	0·0017 s.c.	0·0004 s.c.	0·00086 s.c.	—	0·05 mg s.c.
	Fentanyl	0·02 s.c.	0·034 s.c.	0·0080 s.c.	—	0·5 mg s.c.
Weak narcotic antagonist	Pentazocine	⩾100 s.c.	3·0 s.c.	1·85 s.c.	60·0 s.c.	20–30 mg i.m.
	M5046 hydrochloride	⩾100 s.c.	11·5 s.c.	2·50 s.c.	8·0 s.c.	?
Strong narcotic antagonist	Nalorphine hydrobromide	⩾100 s.c.	2·1 s.c.	5·0 s.c.	0·48 s.c.	15 mg s.c./i.m.
	Levallorphan tartrate	⩾100 s.c.	2·4 s.c.	125·0 s.c.	0·30 s.c.	?
	M285 hydrochloride	⩾100 s.c.	0·028 s.c.	2·3 s.c.	0·013 s.c.	0·5–1·0 mg i.m.
	M5050 hydrochloride	⩾100 s.c.	>100 s.c.	>100 s.c.	0·004 s.c.	?
Tranquillizer/ analgesic	Methotrimeprazine	⩾100 i.m.	0·39 i.m.	>50 i.m.	—	15–30 mg i.m.
	Chlorpromazine	⩾100 i.m.	0·79 i.m.	>50 i.m.	—	0

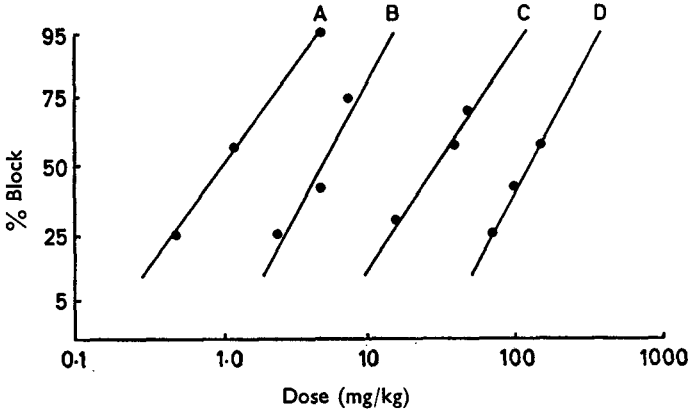

FIG. 2. Dose-response lines obtained with four analgesic drugs in the bradykinin-antagonism test. A. Morphine. B. Nalorphine. C. Phenylbutazone. D. Aspirin. Each point represents the mean response of 5 rats at the time of peak-effect for the analgesic agent. The probit of the proportion of animals in which the bradykinin-induced syndrome is blocked by the analgesic (ordinate) is plotted against logarithm of dose (abscissa). The lines do not deviate significantly from parallelism at the 95% level of confidence.

Weak and strong narcotic analgesics were active in all three tests, the mouse test being the most, and the rat tail-pressure the least sensitive of the three. In all tests, etorphine, fentanyl and morphine were correctly ranked in this descending order of potency. Fentanyl and etorphine appear to be less potent in man, relative to morphine, than might be predicted from the animal test results.

The narcotic antagonists used were consistently inactive in the rat tail-pressure test and, with the exception of M5050 [*N*-(cyclopropylmethyl)-tetrahydro-7(1-hydroxy-1-methlethyl)-6,14-*endo*ethanonororipavine (Bentley, 1967)], active in the Hendershot & Forsaith test. These findings present no novelty but it was of interest to find the antagonists, other than levallorphan and M5050, to be active in the bradykinin-antagonism test. There appears to be no correlation of the potency of these drugs as antagonists of morphine in the rat, and their activity in tests for analgesia; nor can morphine-antagonist activity be related with human analgesic potency where it is known. Thus, for example, the extremely powerful antagonist M5050 appears to be entirely lacking in analgesic activity in the animals, while the very weak antagonist pentazocine is moderately active in both Hendershot & Forsaith and the bradykinin-antagonism tests. Taber & others (1964), Blumberg & others (1965) and Pearl & Harris (1966) reached the same conclusion but found good parallelism between the phenylquinone writhing test and the analgesic potencies reported in man. In general terms we too find the Hendershot & Forsaith test to have good predictive value. It ranks pentazocine, nalorphine and M285 [*N*-(cyclopropylmethyl)tetrahydro-7-(1-hydroxy-1-methylethyl)-6,14-*endo*ethenonororipavine; cyprenorphine hydrochloride

(Bentley, Boura & others, 1965)] in an ascending order of potency, and the values in man follow the same pattern. However, in our experience, levallorphan is almost as potent in the Hendershot & Forsaith test as is nalorphine. This raises an interesting point since Blumberg & others (1965) considered their relatively high ED50 of 25 mg/kg for levallorphan in the Hendershot & Forsaith test to be consistent with the lack of efficacy of this drug as an analgesic in man, quoting Foldes (1964) as their clinical authority. However, in a more recent evaluation in man, Keats & Telford (1966) found 8 mg of levallorphan to be almost equipotent with 10 mg of morphine and hence, by analogy, equipotent also with nalorphine (Lasagna, 1964) as predicted by our results from the Hendershot & Forsaith test.

Since levallorphan is only weakly active as an antagonist of bradykinin in the rat (ED50 125 mg/kg) the predictive value of this test in the antagonist-analgesic group of drugs cannot be considered assured. Much depends on the outcome of scheduled trials in man with M5046 [*N*-cyclopropylmethyl)tetrahydro-7-(1-hydroxy-1-methylethyl)-6,14-*endo*ethanonorthebaine (Bentley, 1967)], which by prediction from the animal test should be active but less potent than pentazocine, and M5050 which appears to be a highly specific antagonist without analgesic activity, as well as the results of Keats and Telford's further studies with levallorphan which are in progress.

We were unable to distinguish between chlorpromazine, which lacks analgesic activity in man, and methotrimeprazine which is reported to have about half the potency of morphine (Montilla, Frederik & Cass, 1963; Pearson & De Kornfeld, 1963; Lim, Miller, Guzman & others, 1966). Both drugs were active in the abolition of phenylquinone-induced writhing of mice but without effect in either rat tail-pressure or the bradykinin-antagonism tests.

In predicting the action in man from that in animals, no special advantage can be claimed for the bradykinin-antagonism test in the rat when compared with the mouse phenylquinone-induced writhing test. The area of particularly uncertain parallelism covers antagonist-analgesics and phenothiazines where the clinical reports are often contradictory. The preparation of the rats is relatively laborious and requires a degree of skill not called for in the mouse test. However, unlike the nociceptive stimuli usually associated with the production of experimental pain, the bradykinin stimulus is liminal and apparently not injurious. A secondary advantage accruing from this is the opportunity to follow the time-course of an analgesic effect in every animal under test.

The possible relation between bradykinin-induced pseudo-affective responses in animals, and pain in man has been emphasized by Guzman & others (1964) and by Lim, Guzman, Rogers & others (1964). Supporting evidence comes from the work of Burch & De Pasquale (1962), and more recently from that of Coffman (1965). These authors have described the sensations of pain produced by injection of small doses of the nonapeptide into the brachial artery of human volunteers and the block effected by normal doses of analgesic drugs. Also relevant are the investigations of

Keele (1960), on the basis of which he has suggested that locally-released bradykinin may act as a transmitter at visceral pain receptor sites.

Acknowledgements. We wish to express our appreciation to Sandoz Ltd. of Basel for the gift of a sample of purified bradykinin, and to thank Mr. A. L. A. Boura for many constructive discussions.

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