Brit. J. Pharmacol. (1963), **21**, 155–164.

NOCICEPTIVE RESPONSES OF GUINEA-PIGS TO INTRADERMAL INJECTIONS OF BRADYKININ AND KALLIDIN-10

BY

H. O. J. COLLIER AND I. R. LEE

From the Department of Pharmacological Research, Parke, Davis & Co., Hounslow, Middlesex

(*Received April* 4, 1963)

Nine behavioural responses were observed to follow with reasonable frequency intradermal injection into guinea-pigs of strongly anisotonic solutions, which are known to cause pain in human skin. These responses were recorded on a form, and a procedure was devised for obtaining a nociceptive score from the records. Intradermal injection of bradykinin or kallidin-10 gave a response pattern indistinguishable from that to a strongly anisotonic solution. Each kinin raised the nociceptive score in response to the control solutions, as also did acetylcholine and histamine; but 5-hydroxytryptamine and *p*H changes within the range of 3.1 to 10.3 did not significantly raise the control score. The effect of bradykinin was depressed by subcutaneous injection of morphine or codeine, but was unaffected by amidopyrine, calcium acetylsalicylate, chlorpromazine or phenylbutazone.

The work to be described had several aims. One was to determine whether substances known to elicit pain after injection into human skin would evoke a characteristic pattern of response when injected intradermally into a convenient species of laboratory mammal. If they did, it was intended to devise a method of measuring this effect, with a view to predicting from animal experiments the likelihood that new substances would either cause or reduce pain in man. Particular questions of interest were whether bradykinin and kallidin-10 were effective in eliciting such responses in the guinea-pig and, if so, whether this effect was suppressed by acetylsalicylate and related drugs, which in this species antagonize bronchoconstriction induced by bradykinin, but not its other effects (Collier & Shorley, 1960).

METHODS

Most experiments were performed in a small windowless room, free from disturbance, with temperature maintained at 21 to 22° C. After clipping the hair of the back and flanks of guinea-pigs of a white strain with electric shears, the animals were allowed to become accustomed to this room for some hours before treatment. Each animal was placed alone in an observation cage for 90 sec, injected intradermally with 0.05 ml. of test solution and immediately replaced in the cage, where it was observed for 50 sec by two experimenters who recorded its behavioural reactions on a form (Table 1). Test solutions were coded, neither experimenter being aware of the code.

Four to eight sites were injected in each animal, the experimental design being a Graeco-Latin or Latin square, in which animals, order of injection, site of injection and type of treatment were taken into account. In one experiment on nine guinea-pigs a Youden square design was used (Table 3). One cross-over experiment was performed with two groups of four animals in a Latin square design (Table 4).

TABLE 1

RESPONSES OF GUINEA-PIGS TO INTRADERMAL INJECTIONS OF PHOSPHATE BUFFER SOLUTIONS OF DIFFERENT MOLARITIES, TO SHOW METHOD OF SCORING

This is the protocol from one animal in a larger experiment. Volumes of 0.05 ml. of buffer solution at *p*H 6·2 were injected intradermally and, after each injection, the animal was observed alone in a cage for 50 sec. In the " wet dog " response the animal shakes the head and skin of the body. Sites of injection: L, left; R, right; F, front; M, middle; H, hind. Code of solutions: A, 0·025 M; B, 0·4 M; C, 0·1 M; D, 0·05 M; E, 0·2 M; F, 0·0125 M. For undirected responses: P, present; A, absent

Treatment			Responses													
			Directed to site			Directed to skin		Undirected							Nociceptive score	
Order	Code	Site	Bite (no.)	Scratch (no.)	Score	" Wet dog " (no.)	Score	Backing	Kicking	Circling	Rearing	Biting cage	Squeaking	Score	Total score	10log (total score+1)
6	C	LF	0	0	0	0	0	A	P	P	A	A	P	3	3	6
5	E	LM	1	0	2	0	0	A	A	A	P	A	P	2	4	7
2	B	LH	23	0	46	2	2	A	P	P	A	A	P	3	51	17
4	A	RF	4	2	12	2	2	A	P	A	P	A	P	3	17	13
1	F	RM	8	0	16	0	0	A	A	P	A	A	P	2	18	13
3	D	RH	0	0	0	0	0	A	A	A	P	A	P	2	2	5

Nine behavioural responses followed with reasonable frequency injection of strongly anisotonic solutions, which are known to evoke pain on injection into human skin (Lindahl, 1961a). Two of these responses were directed towards the site of injection. These were biting or licking the site and scratching it with the ipsilateral hind leg. One response, in which the head and skin of the body were vigorously shaken (the " wet dog " response), was directed towards the skin as a whole. The remaining responses—backing, kicking, circling, rearing on the hind legs, biting the cage and squeaking—were not directed towards the site of injection or to the skin as a whole. All the foregoing responses will be referred to as " nociceptive responses."

The form used for recording these responses is shown in Table 1, which is the protocol from one animal in a larger experiment. From this record a score was obtained by allotting weight according to the certainty with which a response resulted from the injection. Thus, biting or scratching the site of injection scored 2, and the " wet dog " response scored 1, for each occasion observed. Other responses scored 1 for presence at any time during the observation period. The resulting score was transformed to 10log(score+1), in order to equalize variance at different response levels. The transformed score will be called the " nociceptive score," and it will be used in all Figs. and Tables.

To test the reliability of this scoring system, the results of the three experiments illustrated in Fig. 1 were combined, and the responses directed to the site of injection were analysed separately from other responses. Both groups of responses showed the expected increases with injections of strongly anisotonic solutions; but the " directed group " gave a more pronounced dose/response relationship. This justified including both types of response in the score, while allotting less weight to those responses not directed to the site of injection.

Phosphate buffer, Tyrode and 0.9% saline solutions, as solvents for substances to be injected, when tested each gave base-line scores, which resulted mainly from undirected responses. A control treatment with solvent alone was therefore included in all experiments to give a base-line for reference.

In experiments on *p*H effects, 0.1 M-phosphate buffer solutions (Dawson, Elliott, Elliott & Jones, 1959) were used, the *p*H range being extended by addition of 0.1 M-phosphoric acid or 0.1 M-sodium hydroxide. Values of *p*H were checked by meter.

The following substances were injected intradermally, dissolved in 0.1 M- or, on one occasion, in 0.05 M-phosphate buffer at a *p*H of 6.2: bradykinin, synthesized by Nicolaides & DeWald (1961), the decapeptide kallidin-10 (or II), synthesized by Nicolaides, DeWald & McCarthy (1961), acetylcholine bromide, histamine acid phosphate and 5-hydroxytryptamine creatinine sulphate. Doses of substances injected intradermally were successively varied by a factor of 10. For some days after intradermal injections, animals were examined by eye for gross signs of skin necrosis at the site of injection.

The following substances were administered systemically in saline: amidopyrine, calcium acetylsalicylate (prepared by dissolving soluble aspirin, B.P.), chlorpromazine, codeine phosphate, morphine sulphate and phenylbutazone. Weights of salts are expressed as weights of active acid or base.

RESULTS

Solutions of different p*H and tonicity*

In two experiments, eight 0.1 M-phosphate buffer solutions of *p*H values between 3.1 and 10.3 were injected intradermally. Over this range of *p*H, the mean nociceptive score did not exceed 4, and differences between scores were not significant.

Fig. 1 illustrates three experiments, in each of which six guinea-pigs received intradermal injections of buffer solutions of *p*H 6.2 of various molarities. These experiments were performed on different occasions within a period of 3 months. In all of them, 0.05, 0.1 and 0.2 M-solutions consistently gave low scores, and solutions of lower or higher molarity gave high scores. Since 0.05 M-phosphate buffer is approximately isotonic with 0.3% saline and 0.2 M-buffer with 1.2% saline, the

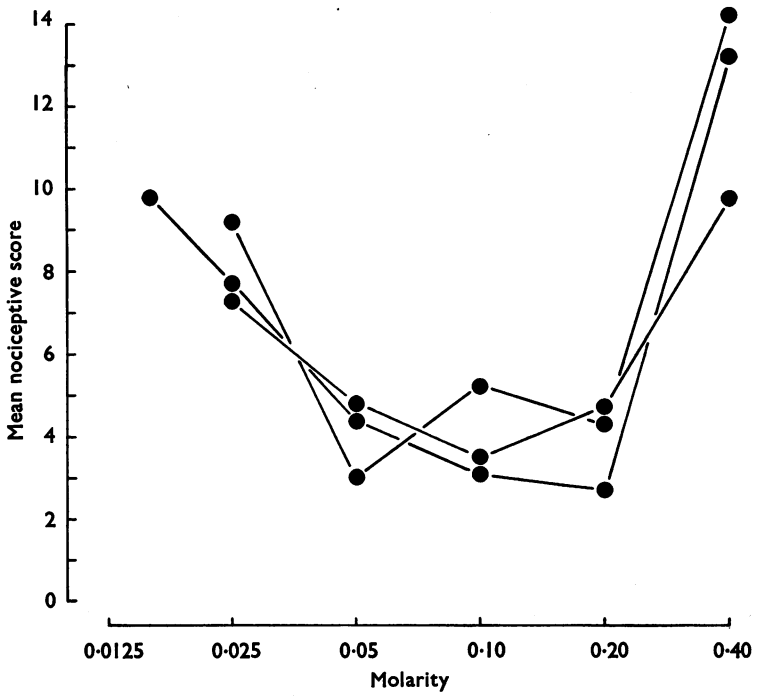

Fig. 1. Relation of nociceptive score to tonicity. Three separate experiments performed within a period of 3 months are illustrated. In each of these, 0.05 ml. volumes of phosphate buffer solutions at *p*H 6.2 of different molarities were injected intradermally. Injections were at six or eight sites into each of six or eight guinea-pigs in a Latin or Graeco-Latin square design. 0.05, 0.1 and 0.2 M-phosphate buffer solutions are approximately isotonic with 0.3, 0.6 and 1.2% salines respectively.

guinea-pigs did not respond vigorously to variations of tonicity within the range one-third to four-thirds isotonic. They were highly responsive, however, to solutions that were one-fifth or three-times isotonic. No skin necrosis was visible for several days after injection.

Bradykinin and kallidin-10

Bradykinin (10 to 1,000 μg/ml. intradermally) elicited a pattern of responses indistinguishable from that to strongly anisotonic buffer. When log concentration of bradykinin was plotted against nociceptive score, a straight line of highly significant slope ($P<0.001$) was obtained (Fig. 2). Fig. 2 shows also that scoring only those responses directed to the site of injection yielded a comparable curve and a lower base-line score.

In three experiments, one of which is illustrated in Table 2, the power of kallidin-10 to elicit nociceptive responses was compared with that of bradykinin. In the experiments shown in this table, kallidin was active at 100 but not at 10 μg/ml., but in the other experiments it was active at 10 μg/ml. also.

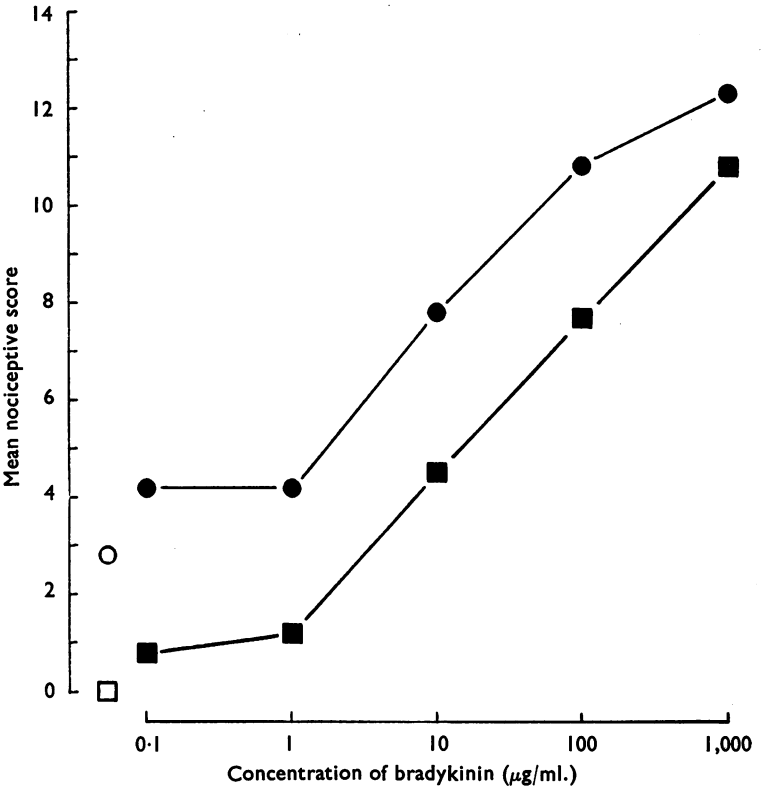

Fig. 2. Dose/response curves for bradykinin. Bradykinin was injected intradermally in 0.05 ml. volumes of 0.1 M-phosphate buffer solution at *p*H 6.2. Injections were at six sites in each of six guinea-pigs in a Latin square design. ●——●, Bradykinin, ○, buffer alone, when all responses were scored; ■——■, bradykinin, □, buffer alone, when only responses directed to the site of injection were scored.

In one experiment with four guinea-pigs, bradykinin was injected at four sites in each animal daily for 5 days, using the same experimental design each day. Here, the scores from corresponding doses of bradykinin increased until the 3rd day and then remained about the same. No other effects differed significantly from day to day. The repeated injections of 1 mg/ml. of bradykinin did not produce any visible skin necrosis.

Histamine, 5-hydroxytryptamine and acetylcholine

In preliminary experiments, in which solutions containing 10 mg/ml. of histamine or 5-hydroxytryptamine were injected intradermally, guinea-pigs showed respiratory distress. These compounds were therefore tested at 1 and 0.1 mg/ml. In the same experiment, acetylcholine and bradykinin were tested at the same concentrations. Table 3 gives the results of an experiment in nine guinea-pigs, in which 5-hydroxytryptamine was not significantly active, but acetylcholine ($P=0.005$) and histamine ($P<0.05$) were significantly more effective than buffer solution. Weight

TABLE 2

COMPARISON OF NOCICEPTIVE SCORES OBTAINED WITH BRADYKININ AND KALLIDIN-10

Eight sites were injected intradermally in each of eight guinea-pigs in a Graeco-Latin square design. Bradykinin and kallidin-10 were given in 0·05 ml. volumes of 0·1 M-phosphate buffer at *p*H 6·2. The last column gives the significance of the responses from those to buffer alone

Substance	Concentration (μg/ml.)	Mean nociceptive score	Significance of difference from buffer
Bradykinin	1,000	6·75	$P<0·001$
	100	4·6	$P<0·005$
	10	3·9	$P<0·025$
	1	0·6	Not significant
Kallidin-10	100	4·9	$P<0·005$
	10	2·0	Not significant
	1	1·75	Not significant
Buffer alone	—	1·0	—

for weight, bradykinin was considerably more active ($P<0.001$) than either histamine or acetylcholine. Histamine produced a few responses directed to the site of injection, but acetylcholine produced none, although it increased the number of undirected responses. On the following day, sites of injection showed no visible necrosis.

Effects of drugs on the response to bradykinin

Six drugs were tested for ability to reduce the nociceptive score of bradykinin. Morphine (5 and 10 mg/kg subcutaneously), given 30 min before bradykinin, reduced both the bradykinin score and the background score from buffer solution alone. This result is shown in Fig. 3, based on an experiment in three groups of four animals. Codeine (40 mg/kg by the same route), 20 min before bradykinin, had a similar effect in a cross-over experiment in two groups of four animals (Table 4). Amidopyrine (160 mg/kg intraperitoneally), calcium acetylsalicylate (200 to 800 mg/kg intraperitoneally), chlorpromazine (4 to 64 mg/kg subcutaneously) and phenylbutazone (50 to 200 mg/kg subcutaneously) failed to reduce the nociceptive score of bradykinin.

TABLE 3

COMPARISON OF NOCICEPTIVE SCORES OBTAINED WITH ACETYLCHOLINE, HISTAMINE, 5-HYDROXYTRYPTAMINE AND BRADYKININ

Eight sites were injected intradermally in each of nine guinea-pigs in a Youden square design. Other details as in Table 2

Substance	Concentration (μg/ml.)	Mean nociceptive score	Significance of difference from buffer	Significance of difference from bradykinin
Acetylcholine	1,000	4·3	$P=0·005$	$P<0·001$
	100	4·4		
Histamine	1,000	3·2	$P<0·05$	$P<0·001$
	100	4·2		
5-Hydroxy-tryptamine	1,000	3·1	Not significant	$P<0·001$
	100	2·5		
Bradykinin	1,000	8·0	$P<0·001$	—
	100	5·9		
Buffer alone	—	1·8	—	$P<0·001$

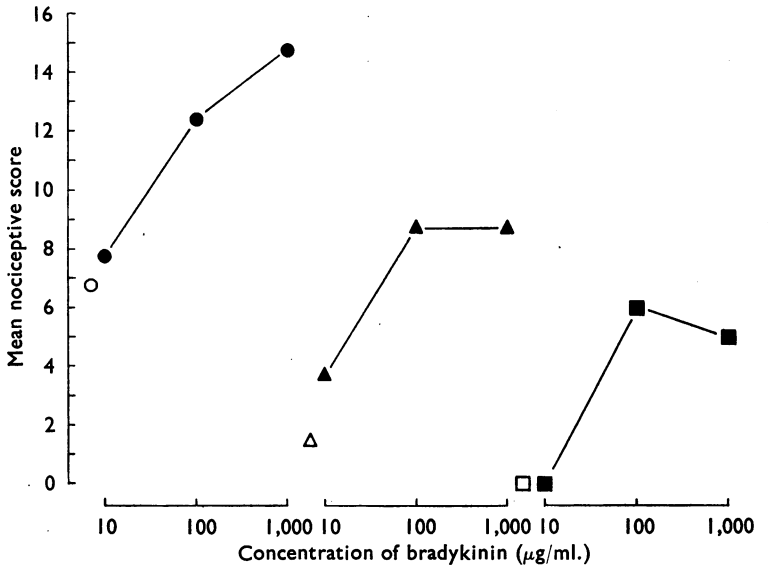

Fig. 3. Depression by morphine of nociceptive responses to bradykinin. In each of three experiments, four sites were injected intradermally with bradykinin in each of four guinea-pigs in a Graeco-Latin square design. Morphine sulphate was administered subcutaneously 30 min before bradykinin was injected in 0.05 ml. volumes of 0.05 M-phosphate buffer solution at *p*H 6.2. ●——●, Bradykinin, and ○, buffer, without morphine; ▲——▲, bradykinin, and △, buffer, after morphine (5 mg/kg); ■——■, bradykinin, and □, buffer, after morphine (10 mg/kg).

Effects of position and order of injection

The results of twelve experiments involving seventy guinea-pigs were examined statistically for the effects upon nociceptive score of position and order of intradermal injection. There was no difference between scores of responses to injections on the left and right sides, but sensitivity declined from front to rear of the animal. Sensitivity increased with repeated injections. Both these effects were slight compared with differences between animals.

Intraperitoneal injection of hypertonic saline and of bradykinin

Lewis (1942) distinguished the effects of stimulating superficial and deep nociceptors in spinal animals. We therefore compared the reactions of guinea-pigs to intraperitoneal injections of a hypertonic solution and of bradykinin with those to intradermal injection described above. Intraperitoneal injection of 0.1 to 0.4 ml. of 5% saline usually elicited one or more of three responses: (1) hollowing and hunching of the back; (2) sitting on the haunches; and (3) lying on one side. Injection of similar volumes of 0.9% saline containing 100 μg/ml. of bradykinin provoked the first two, but not the third of these responses. Instead, animals were active and exhibited the kicking and squeaking responses seen after intradermal injection of bradykinin; these might have been caused by contamination with bradykinin of the skin wound made by the needle. All responses disappeared within 1 min after injection. Saline (0.9%) produced none of these responses.

TABLE 4

SUPPRESSION BY CODEINE OF NOCICEPTIVE RESPONSES TO BRADYKININ

Four sites were injected intradermally in each of two groups of four animals on two successive days in a Latin square cross-over design. Codeine phosphate was administered subcutaneously 20 min before intradermal bradykinin. Details of intradermal injections as in Table 2. Between Day 1 and Day 2 one animal from Group 2 died

Treatment		Mean nociceptive scores	
Codeine (mg/kg)	Bradykinin (μg/ml.)	Day 1 Group 1	Day 2 Group 2
None	1,000	6·0	7·0
	100	6·0	6·0
	10	4·1	5·3
	Buffer alone	3·5	2·7
		Group 2	Group 1
40	1,000	2·0	1·0
	100	0·8	1·0
	10	0·8	0
	Buffer alone	0·8	0

DISCUSSION

Sherrington (1903) showed that a scratch reflex could be elicited in the spinal dog either by noxious pressure or by light stroking of the skin. The question therefore arises whether the responses to intradermal injection observed in the guinea-pig were nociceptive or tangioceptive. Lindahl (1961b) found that sodium chloride solutions of different molarities, administered intracutaneously by jet-injector to human volunteers, gave intensities of pain that were lowest for concentrations between 0.09 and 0.15 M, and increased by two- to three-fold when molarity was raised or lowered much beyond this range. The guinea-pig responded to similar changes in tonicity, except that the range of molarities giving minimal score was slightly wider. The parallelism in the effectiveness of tonicity changes in man and guinea-pig supports the view that the responses of the guinea-pig to strongly anisotonic solutions were mainly nociceptive. Three facts suggest that the responses to intradermal injection of bradykinin were also nociceptive. First, the pattern of responses to bradykinin was indistinguishable from that to strongly anisotonic solutions; second, bradykinin causes pain in man (Table 5); and third, the responses of guinea-pigs were suppressed by strong analgesics (Fig. 3, Table 4), but not by weak analgesics or by anti-inflammatory agents or tranquillizers.

Intradermal injection of 0.1 M-phosphate buffer, 0.9% saline or Tyrode solution produced a base-line score, which resulted mainly from undirected responses. Since this score was higher than that obtained from uninjected animals left undisturbed in a cage, it presumably arose from the stimuli associated with injection. The fact that morphine (Fig. 3) and codeine (Table 4) suppressed this base-line score suggests that it too was derived mainly from nociceptive responses. Presumably, therefore, the responses to kallidin-10, histamine and acetylcholine, which yielded significantly higher scores than buffer solution, were also nociceptive, although this possibility was not tested with analgesic drugs.

A slightly hypotonic solution was chosen as solvent for substances to be injected intradermally, because it fell in the middle of the range of tonicity in which the

score was minimal, and because it allowed substances to be dissolved without reaching noxious levels of hypertonicity.

The scoring system adopted, which was based on the principle that weight should be allotted according to the degree of certainty that the response resulted from action of the solution injected intradermally, was justified by the analysis in separate groups of responses directed to the site of injection and of all other responses in several series of experiments. When only directed responses were counted, anisotonic solutions and bradykinin (Fig. 2) gave sharp dose/response relationships and buffer solution gave low base-line scores, but acetylcholine was inactive. Inclusion of all other responses also yielded dose/response relationships, although these were less pronounced and base-line scores were higher, and activity was shown by acetylcholine. Scoring all responses and giving weight to directed ones appeared to take best advantage of the information obtained.

How far do the abilities of endogenous substances to evoke nociceptive responses in the guinea-pig compare with those in other species ? Table 5, which compares

TABLE 5

MINIMAL DOSES OF SOME ENDOGENOUS SUBSTANCES ELICITING PAIN OR NOCICEPTIVE RESPONSES IN MAN, DOG AND GUINEA-PIG

* Elliott, Horton & Lewis, 1961. § Armstrong, personal communication. † Armstrong *et al.*, 1953. ‡ Herxheimer, personal communication. || Lindahl, 1961c. ¶ Guzman, Braun & Lim, 1962

Substance	Human skin		Dog ¶	Guinea-pig
	On blister base (μg/ml.)	Injection (μg/ml.)	Various sites of injection (μg)	intradermal injection (μg/ml. and actual dose in μg)
Bradykinin	0·1–1*	100‡	0·1–6·3	10 (0·5)
Kallidin-10	1§	—	—	10 (0·5)
Acetylcholine	10–100†	1,000||	250–1,000	100–1,000 (5–50)
Histamine	1,000†	100||	25–300	100–1,000 (5–50)
5-Hydroxy-tryptamine	0·1–100†	100–1,000||	25–200	>1,000 (>50)

results obtained by several authors in man and dog with those we have described, shows that the activities of substances tested in the guinea-pig, except 5-hydroxytryptamine, resemble those in other species. Although in man buffers of very high or low *p*H elicit pain on intracutaneous injection (Lindahl, 1961d), acid solutions of *p*H>3 were not painful when applied to human blister-bases (Armstrong, Dry, Keele & Markham, 1953). The failure of acidic buffer solutions of *p*H>3 to increase nociceptive scores in the guinea-pig is consistent with the finding of Armstrong *et al.*

The finding that morphine and codeine, but not amidopyrine or acetylsalicylate, suppressed the nociceptive response to intradermal bradykinin might form the basis of a method for testing potential narcotic analgesic drugs. However, the procedure needed to determine the effective dose of a drug would be less economical than that in the multiple toe-pinch test (Collier, Warner & Skerry, 1961) which shows a similar sensitivity to drugs.

The failure of acetylsalicylate and amidopyrine to block the action of bradykinin on nociceptors in guinea-pig skin is consistent with the failure of these drugs to show either specific antagonism of bradykinin wealing (Collier & Shorley, 1960) or antinociceptive action in the multiple toe-pinch test (Collier *et al.*, 1961). The failure of acetylsalicylate to suppress nociceptive responses to intradermal injection of bradykinin into the guinea-pig contrasts with its success in suppressing those to intra-arterial injection of bradykinin into the dog (Braun, Guzman, Horton, Lim & Potter, 1961) and to intraperitoneal injection of noxious agents into the mouse (Siegmund, Cadmus & Lu, 1957; Hendershot & Forsaith, 1959). Lewis (1942) expressed the view that nociception from skin differs in quality from that arising from deeper structures. His view is supported by the differences in the reactions of guinea-pigs to the intradermal and the intraperitoneal injections of hypertonic solutions. The contrast between the ineffectiveness of acetylsalicylate when noxious agents are applied to skin and its effectiveness when they are applied to internal structures may express another difference between deep and superficial nociception.

We wish to thank Dr B. T. Warner, Mr A. R. Hammond and Mr L. C. Dinneen for providing the statistical designs and analyses, Mr L. C. Dinneen for help with the computation and illustrations and Miss G. C. Clarke, Miss S. Horwood-Barrett and Mr M. A. Palmer for technical assistance. It is a pleasure to acknowledge helpful discussions with Professor C. A. Keele and Dr M. Schachter. Mr A. J. Middleton kindly determined the depression of freezing point of buffer solutions.

REFERENCES

ARMSTRONG, D., DRY, R. M. L., KEELE, C. A. & MARKHAM, J. W. (1953). Observations on chemical excitants of cutaneous pain in man. *J. Physiol.* (*Lond.*), **120**, 326–351.

BRAUN, C., GUZMAN, F., HORTON, E. W., LIM, R. K. S. & POTTER, G. D. (1961). Visceral receptors, pain, bradykinin and analgesic agents. *J. Physiol.* (*Lond.*), **155**, 13–14*P*.

COLLIER, H. O. J. & SHORLEY, P. G. (1960). Analgesic antipyretic drugs as antagonists of bradykinin. *Brit. J. Pharmacol.*, **15**, 601–610.

COLLIER, H. O. J., WARNER, B. T. & SKERRY, R. J. (1961). A multiple toe-pinch method for testing analgesic drugs. *Brit. J. Pharmacol.*, **17**, 28–40.

DAWSON, R. M. C., ELLIOTT, D. C., ELLIOTT, W. H. & JONES, K. M. (1959). Ed. *Data for Biochemical Research*, p. 200. Oxford: University Press.

ELLIOTT, D. W., HORTON, E. W. & LEWIS, G. P. (1961). Actions of pure bradykinin. *J. Physiol.* (*Lond.*), **153**, 473–480.

GUZMAN, F., BRAUN, C. & LIM, R. K. S. (1962). Visceral pain and the pseudaffective response to intra-arterial injection of bradykinin and other algesic agents. *Arch. int. Pharmacodyn.*, **136**, 353–384.

HENDERSHOT, L. C. & FORSAITH, J. (1959). Antagonism of the frequency of phenylquinone-induced writhing in the mouse by weak analgesics and non-analgesics. *J. Pharmacol. exp. Ther.*, **125**, 237–240.

LEWIS, T. (1942). *Pain*, pp. 36–46. New York: Macmillan.

LINDAHL, O. (1961). Experimental skin pain induced by injection of water-soluble substances in humans. *Acta physiol. Scand.*, **51** (Suppl. 179), (a) p. 5, (b) pp. 38–41, (c) pp. 48–50, (d) pp. 51–58.

NICOLAIDES, E. D. & DEWALD, H. A. (1961). Studies on the synthesis of polypeptides. Bradykinin. *J. org. Chem.*, **26**, 3872–3876.

NICOLAIDES, E. D., DEWALD, H. A. & MCCARTHY, D. A. (1961). The synthesis of a biologically active decapeptide having the structure proposed for Kallidin II. *Biochem. biophys. Res. Commun.*, **6**, 210–212.

SHERRINGTON, C. S. (1903). Qualitative difference of spinal reflex corresponding with qualitative difference of cutaneous stimulus. *J. Physiol.* (*Lond.*), **30**, 39–46.

SIEGMUND, E., CADMUS, R. & LU, G. (1957). A method for evaluating both non-narcotic and narcotic analgesics. *Proc. Soc. exp. Biol.* (*N.Y.*), **95**, 729–731.