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THE ABDOMINAL CONSTRICTION RESPONSE AND ITS SUPPRESSION BY ANALGESIC DRUGS IN THE MOUSE

BY

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After intraperitoneal injection of a noxious agent, the rat and the mouse show a response consisting of a wave of constriction and elongation passing caudally along the abdominal wall, sometimes accompanied by twisting of the trunk and followed by extension of the hind limbs (Vander Wende & Margolin, 1956; Siegmund, Cadmus & Lu, 1957). This response has been variously called "writhing" (Vander Wende & Margolin, 1956), "stretching" (Koster, Anderson & de Beer, 1959), "cramping" (Murray & Miller, 1960) and "squirming" (Whittle, 1964a). Because of the emotional implications of these terms, it was later called the "abdominal constriction response" (Collier, Hammond, Horwood-Barrett & Schneider, 1964). We describe here an examination of the ability of a number of substances, including some occurring in tissues and causing pain in man (Keele & Armstrong, 1964), to elicit abdominal constriction responses in mice.

Vander Wende & Margolin (1956) and Siegmund *et al.* (1957) showed that codeine, morphine and pethidine, given subcutaneously, and procaine, given intraperitoneally, suppressed the abdominal constriction response. They concluded that the response was nociceptive and they used it as a basis for testing analgesic drugs. The antinociceptive tests developed from this work have the advantage that they are sensitive to antipyretic drugs (Siegmund *et al.*, 1957; Hendershot & Forsaith, 1959; Koster *et al.*, 1959; Keith, 1960) and to narcotic antagonists (Taber, Greenhouse & Irwin, 1964; Blumberg, Wolf & Dayton, 1965; Pearl & Harris, 1966). These tests have, however, three disadvantages: (1) exposure of animals to peritoneal stimulation intense enough to induce repeated abdominal constrictions for longer than necessary is ethically undesirable and needlessly time consuming; (2) increased permeability of blood vessels accompanies peritoneal irritation (Northover, 1963, 1964) and the development of abdominal constrictions (Whittle, 1963, 1964a and b), and so an antinociceptive effect cannot easily be distinguished from an anti-inflammatory; and (3) the test produces many false positives (Hendershot & Forsaith, 1959; Emele & Shanaman, 1963; Brittain, Lehrer & Spencer, 1963; Okun, Liddon & Lasagna, 1963). We describe new forms of abdominal constriction test in the mouse, in which some of these disadvantages are lessened. Preliminary accounts of this work have been published (Collier *et al.*, 1964; Collier, James & Schneider, 1965).

METHODS

Table 1 gives the substances tested for ability to elicit the abdominal constriction response. Inorganic salts, acetic acid and ethanol were dissolved in distilled water; 1:4-phenylbenzoquinone

was dissolved in 5% w/v ethanol in water; other substances were dissolved in 0.9% w/v sodium chloride in water or suspended in 20% w/v gum acacia in water. The arachidonic acid was grade II, 90–95% (Sigma). The betanin was prepared from beetroot by Watson (1964). Two samples of synthetic bradykinin were used: a pure solid prepared by Nicolaides & De Wald (1961) and a solution in ampoules containing bradykinin 100 μg and chlorbutol 5 mg/ml. of a sodium acetate-acetic acid buffer (Sandoz). Unless otherwise stated, the solid bradykinin is referred to. Mustard flour (Colman), 2.5% w/v suspension in water, was used. The slow-reacting substance in anaphylaxis (SRS-A) was a charcoal purified specimen, prepared by the method outlined by Berry & Collier (1964). The SRS-A control was prepared in exactly the same way, except that lungs from non-sensitized guinea-pigs were used. Table 5 gives the drugs tested for ability to suppress nociceptive responses. Drugs were dissolved in water, or in sodium chloride 0.9% w/v in water or suspended in gum acacia 20% w/v in water. Doses of organic salts are expressed throughout as weights of active acid or base/kg body-weight; doses of inorganic salts are given as concentrations of the solution administered. All substances were administered in a dose volume of 10 ml./kg body-weight.

Experiments were carried out at a laboratory temperature of 21°–25° C, in male albino mice. Food and water were withheld during experiments. Solutions tested for ability to elicit abdominal constriction responses were injected intraperitoneally at laboratory temperature into mice of T.O. strain, randomized and observed in groups of five. After challenge, the mice were placed in a translucent plastic box and their responses counted during continuous observation for 2, 10 or 30 min.

Drugs to be tested for ability to suppress abdominal constriction responses were administered subcutaneously or orally 20 min before intraperitoneal injection of acetylcholine bromide (3.2 mg/kg), adenosine-5′-triphosphate (4.1 mg/kg), bradykinin (0.4 mg/kg), potassium chloride (2.5% w/v in water) or tryptamine hydrochloride (20 mg/kg). Responses were counted for 2 min afterwards. In tests with acetylcholine, to exclude the possibility that an apparent antinociceptive effect was due to specific antiacetylcholine activity in the test drug, a non-lethal dose of up to 8×ED50 of the test drug was injected subcutaneously 20 min before a dose of physostigmine sulphate (2.0 mg/kg subcutaneously), estimated from experiments in 180 mice to kill 95% of animals (LD95). Twenty minutes after physostigmine, the proportion of deaths among mice receiving the test drug was compared with that among those not receiving the test drug. A control group was given the same dose of test drug alone.

To test disco-ordination, after antinociceptive tests, animals were placed on the surface (1/10 in. square wire mesh) of a drum, of 12 in. diameter, rotating at 1 rev/min (Collier, Hall & Fieller, 1949; Collier, 1964). In some disco-ordination tests, a derivative of the T.O. strain—Tuck's T.T. strain—was used.

An animal was taken as responding to an intraperitoneal challenge when at least one abdominal constriction was observed. The significance of differences between the proportions of animals responding to different treatments was found by the chi-squared test. A value of $P<0.05$ was taken as significant; where higher degrees of significance were obtained, the P value is stated. From the quantal results in abdominal constriction and other tests, the median effective dose (ED50) of an antinociceptive drug and its 95% fiducial limits were calculated by logit analysis (Finney, 1964). When less than 90% of control animals responded to the challenge substance, a correction was made for "natural mortality" (Finney, 1962).

RESULTS

Responses to intraperitoneal challenge

Table 1 gives the cumulative percentage of mice showing at least one abdominal constriction response at various times after control procedures or after intraperitoneal injection of vehicles or of challenge substances. Animals not injected at all showed no responses within a 30 min period; but those receiving a sham injection, without fluid being introduced into the peritoneal cavity, gave a few responses within 10 min and more within 30 min. Acacia, distilled water, ethanol and sodium chloride 0.9% w/v in water, which were used as vehicles, elicited some responses. The solution of sodium chloride

TABLE 1

DEVELOPMENT OF ABDOMINAL CONSTRICTION RESPONSES IN THE MOUSE AFTER CONTROL PROCEDURES OR AFTER INTRAPERITONEAL INJECTION OF VEHICLES OR CHALLENGE SUBSTANCES

Doses of inorganic salts are expressed as the concentration (w/v) of the total salt in water ; doses of other substances are expressed as mg/kg of active acid or base. The number of mice is the total of animals observed for at least 2 min, but not necessarily over the full 30 min period. N.O., not observed. A value of $P<0{\cdot}05$ is taken as significant. * Significantly more responses than to vehicle. † Significantly more responses than to no injection. ‡ Significantly more responses than to 0·9% sodium chloride solution. § Significantly less responses than to vehicle. DMPP is 1,1-dimethyl-4-phenylpiperazinium iodide (Chen, Portman & Wickel, 1951).

Control procedure, vehicle or challenge substance	Intraperitoneal dose		No. of mice	Cumulative % of mice showing a response by :				
	% w/v	mg/kg		10 sec	30 sec	2 min	10 min	30 min
No injection	—	—	15	0	0	0	0	0
Sham injection	—	—	35	0	0	0	14	23†
Acacia	20	—	20	0	15‡	45‡	70‡	80‡
Distilled water	—	—	40	5	8‡	33‡	38‡	45‡
Ethanol	5·0	—	20	0	0	0	15	55‡
Sodium chloride	0·9	—	245	0	0	2	13	23
Acetic acid	—	50	75	0	0	12	75*	84*
Acetylcholine Br	—	0·4	30	3	6	13	20	67*
	—	0·8	30	3	20*	70*	74*	87*
	—	1·6	40	5	13*	N.O.	N.O.	N.O.
	—	3·2	1,060	1	20*	91*	94*	95*
Pyrophosphate Na	—	2·0	30	0	0	0	N.O.	N.O.
	—	3·9	20	0	0	0	N.O.	N.O.
Adenosine monophosphate Na	—	4·1	30	0	0	17*	50*	57*
Adenosine diphosphate Na	—	2·05	20	0	0	65*	75*	75*
	—	4·1	30	0	0	37*	67*	73*
	—	8·2	20	0	0	65*	80*	80*
Adenosine triphosphate Na	—	2·05	15	0	0	73*	87*	87*
	—	4·1	270	0	10	73*	78*	85*
	—	8·2	100	0	6*	67*	83*	86*
Adenosine tetraphosphate Na	—	4·1	30	0	7	77*	100*	100*
Angiotensin	—	10	20	0	0	10	15	40
Arachidonic acid	—	5	20	0	0	65*	70*	70*
Aspirin	—	56·2	20	0	0§	0§	0§	5§
Aspirin Ca	—	30·4	30	0	0	0	0§	0§
Betanin	—	20	10	0	0	10	100*	100*
Bethanechol Cl	—	1·25	10	0	0	10	40	50
Bradykinin	—	0·06	105	2	26*	45*	N.O.	N.O.
	—	0·4	349	10*	65*	74*	85*	88*
" (Sandoz)	—	0·125	20	10*	30*	45*	50*	60*
Calcium chloride	1·8	—	35	0	0	29	71*	83*
Carbachol Cl	—	1	20	0	30*	55*	55*	55*
Chlorbutol	—	6·25	80	0	1	4	20	35
	—	50	20	0	0	15	55*	70*
DMPP	—	10	25	0	0	0	0	N.O.
Histamine	—	2·5	30	0	3	10	40*	40
acid phosphate	—	10	20	10*	15*	30*	55*	65*
5-Hydroxytryptamine creatinine sulphate	—	0·78	20	10*	10*	40*	60*	80*
5-Hydroxytryptophan	—	100	20	5	5	25*	80*	85*
Magnesium sulphate	2	—	10	0	0	100*	100*	N.O.
Mefenamate Na	—	14·8	10	0	0	0	0	10
Morphine sulphate	—	0·49	10	0	10	10	10	10
Mustard flour	2·5	—	20	20	40*	50	55	N.O.
Nicotine acid tartrate	—	4·0	45	22*	40*	40*	55*	N.O.
1 : 4-Phenylbenzoquinone	—	1	20	0	0	5	100*	100*
	—	2	10	0	0	0	100*	100*
Potassium chloride	0·625	—	30	7	37	53	53	N.O.
	1·25	—	160	17	41	57	77	N.O.
	2·5	—	250	25	53*	73*	76*	N.O.
Potassium sulphate	2·9	—	29	24*	79*	93*	93*	N.O.
Sodium chloride	1·8	—	20	0	0	5	10	15
	4·0	—	20	30*	35*	55*	60*	60*
SRS-A	—	5	25	0	0	4	16	64*
SRS-A control	—	5	25	0	0	5	16	88*
Tremorine HCl	—	10	10	0	10	10	10	N.O.
Tryptamine HCl	—	10	40	0	5	68*	75*	83*
	—	20	310	0	32*	84*	80*	88*
Tryptophan	—	100	15	0	0	0	27	40*

0.9% w/v in water was not significantly more effective in eliciting responses than was a sham injection; but acacia, distilled water and ethanol were significantly more effective than sodium chloride 0.9% w/v in water. A solution of sodium chloride 1.8% w/v in water was not significantly more effective than the 0.9% solution.

Of the challenge substances in Table 1, bradykinin, histamine, 5-hydroxytryptamine, nicotine, potassium sulphate and sodium chloride 4.0% w/v in water caused significantly more animals to respond within 10 sec of injection than did the vehicle. Within 30 sec, acetylcholine, adenosine triphosphate, carbachol, mustard, potassium chloride and tryptamine were significantly effective. Within 2 min of injection, adenosine monophosphate, diphosphate and tetraphosphate, arachidonic acid, 5-hydroxytryptamine and magnesium sulphate were also significantly effective. Still later, acetic acid, betanin, calcium chloride, chlorbutol, phenylbenzoquinone, SRS-A, SRS-A control and tryptophan produced a significant incidence of abdominal constrictions.

Table 2 gives the percentage of mice showing an abdominal constriction response within each 2 min period for up to 10 min after intraperitoneal injection of acetylcholine, adenosine triphosphate, bradykinin, potassium chloride or tryptamine. This table shows that, after 2 min, the percentage of mice responding to acetylcholine, bradykinin and potassium chloride declined; after 4 min, fewer animals responded to adenosine triphosphate and tryptamine also.

TABLE 2

INCIDENCE OF RESPONSES DURING SUCCESSIVE PERIODS AFTER INTRAPERITONEAL INJECTION OF SOME CHALLENGE SUBSTANCES

Details as in Table 1.

Challenge substance	Dose		No. of mice	% of mice showing a response during :				
	% w/v	mg/kg		0–2 min	2–4 min	4–6 min	6–8 min	8–10 min
Distilled water	—	—	35	34	14	9	6	3
Sodium chloride	0·9	—	205	2	5	4	3	5
Acetylcholine	—	3·2	70	87	64	27	14	7
Adenosine triphosphate	—	4·1	40	63	63	35	18	10
Bradykinin	—	0·4	65	75	6	6	9	11
Potassium chloride	2·5	—	45	67	4	0	0	0
Tryptamine	—	20	30	73	60	30	0	7

From the proportions of mice responding within 2 min to various intraperitoneal doses of the challenge substances, after pooling results of all appropriate experiments, the ED50 values of acetylcholine, potassium chloride and tryptamine were computed (Table 3). The dose-response curves of adenosine triphosphate and bradykinin did not allow ED50 values and limits to be computed; the approximate ED50 values are given in Table 3. The challenge doses of these substances used in the antinociceptive tests below were those found to induce abdominal constrictions in approximately the highest percentage of mice. Table 3 also gives these challenge doses and the percentage of mice responding within 2 min to each.

To determine whether the percentage of responses changed when acetylcholine was injected repeatedly at intervals of 20 min, five experiments were performed, each in ten mice. In these, the percentage of animals responding within 2 min to each of three successive injections of acetylcholine remained constant. The pooled values were: first injection, 92%; second injection, 94%; third injection, 92%.

TABLE 3

MEDIAN EFFECTIVE DOSES (ED50) AND CHALLENGE DOSES OF SOME SUBSTANCES THAT RAPIDLY ELICIT ABDOMINAL CONSTRICTIONS

The challenge dose was that used in antinociceptive tests. Doses are expressed in mg/kg, except that of potassium chloride, which is expressed as a percentage solution. Other details as in Table 1.

	Median effective dose		Challenge dose		
Challenge substance	No. of mice	ED50 with 95% limits	Dose	No. of mice	% of mice responding within 2 min
Acetylcholine	1,325	0·763 (0·586–0·928)	3·2	1,060	90·8
Adenosine triphosphate	695	*ca.*1	4·1	350	79·4
Bradykinin	723	*ca.*0·1	0·4	394	70·8
Potassium chloride	934	0·795 (0·476–1·06)	2·5	564	70·0
Tryptamine	550	4·41 (3·04–5·70)	20	330	83·3

Because acetylcholine was chosen as challenge substance for routine antinociceptive tests, its median lethal dose in the T.O. strain of mice was determined, using fifty animals. The LD50 in mg/kg intraperitoneally (with 95% fiducial limits) was 154 (121–185).

Antinociceptive effects

An experiment was performed in eighty mice, over a period of 30 min, to compare the effectiveness of aspirin against abdominal constriction responses induced by bradykinin or by sodium chloride 0.9% w/v in water. Forty mice received calcium aspirin (50 mg/kg subcutaneously) and 20 min later these and forty controls were injected intraperitoneally with either sodium chloride 0.9% w/v in water or with a challenge dose of bradykinin (0.4 mg/kg). Responses were counted continuously in all groups for 30

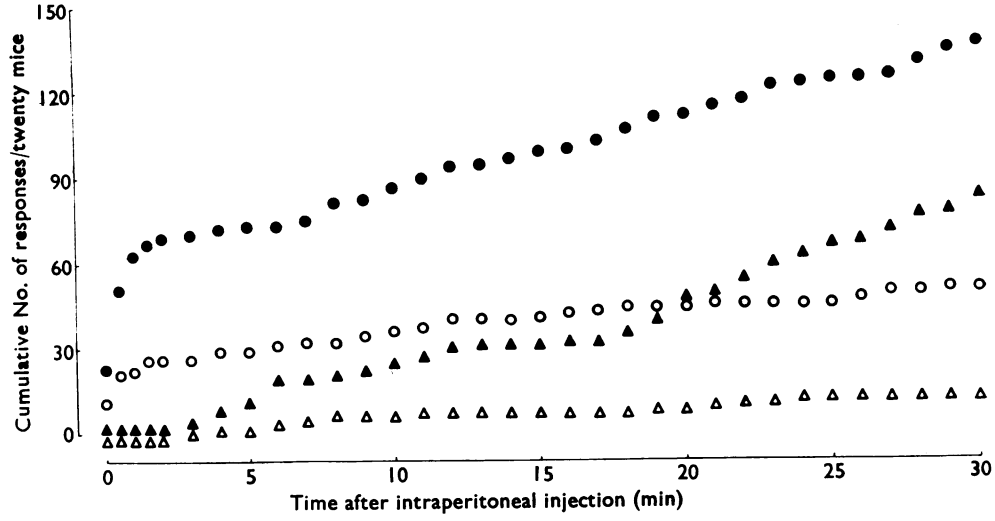

Fig. 1. Effect of aspirin on the incidence of abdominal constriction responses to intraperitoneal bradykinin (0.4 mg/kg) or sodium chloride 0.9% w/v in water, in the mouse. Calcium aspirin (50 mg/kg) or sodium chloride 0.9% w/v in water was injected subcutaneously 20 min before intraperitoneal challenge. ●, sodium chloride subcutaneously and bradykinin intraperitoneally; ○, aspirin subcutaneously and bradykinin intraperitoneally; ▲, sodium chloride subcutaneously and intraperitoneally; △, aspirin subcutaneously and sodium chloride intraperitoneally.

min after intraperitoneal injection. In Fig. 1, the cumulative responses are plotted against time. In this experiment, aspirin effectively reduced the number of responses to bradykinin or to sodium chloride 0.9% w/v in water, during the period when responses to either agent occurred.

This experiment suggests that, in antinociceptive tests, responses may be counted from the time of injection of suitable challenge substances. We therefore tested the ability of representative drugs of the narcotic, narcotic antagonist and antipyretic classes to reduce the incidence of abdominal constriction responses within 2 min of intraperitoneal challenge with acetylcholine, adenosine triphosphate, bradykinin, potassium chloride or tryptamine. Table 4 gives the ED50 values, with fiducial limits where obtainable, and the slopes of the dose-response lines derived from these experiments.

All the drugs in Table 4, except paracetamol, were effective against abdominal constrictions induced by several challenge substances. Morphine was consistently more potent than codeine; and cyclazocine was more potent than any other analgesic drug in Table 4. Aspirin was very significantly ($P<0.001$) more potent against acetylcholine than against adenosine triphosphate, bradykinin, potassium chloride or tryptamine. Differences were also seen in the potencies of other antipyretic drugs against the various challenge substances; but the patterns of potency differed from that of aspirin. Within the dosage range tested, paracetamol was active only against acetylcholine. Whereas, in Table 4, the ratio of potency against acetylcholine to that against tryptamine did not significantly exceed unity with the narcotic and narcotic antagonist drugs, this ratio was never less than 4 for the antipyretic drugs.

Analgesic drugs and drugs of several other classes were tested for ability to inhibit abdominal constrictions induced by intraperitoneal acetylcholine. They were also tested, at a comparable time after treatment, for ability to disco-ordinate mice placed on the rotating drum. Table 5 gives the values of the median effective doses of suppression of abdomnal constrictions (ED50) and for disco-ordination (FD50). This table shows that drugs of the narcotic analgesic, narcotic antagonist, antipyretic, antiacetylcholine, antihistaminic and sympathominetic classes were effective against intraperitoneal challenge with acetylcholine at a dose considerably lower than the disco-ordinating dose.

In the test for protection against physostigmine lethality, atropine, benactyzine, chlorpheniramine, chlorpromazine, diphenhydramine, hydroxyzine, hyoscine, mepyramine, imipramine and pempidine significantly reduced the proportion of mice killed. Except pentazocine, other drugs in Table 5, which were effective against abdominal constrictions induced by acetylcholine, did not significantly lessen physostigmine toxicity. Pentazocine, however, was significantly more effective against adenosine triphosphate or bradykinin than against acetylcholine; whereas atropine showed much less activity against abdominal constrictions induced by bradykinin, potassium chloride or tryptamine, than against those induced by acetylcholine (Table 4).

Since Winter & Flataker (1965) showed that counter-irritants may have apparent antinociceptive effects, acetic acid, mustard and phenylbenzoquinone were tested for ability to suppress the abdominal constriction response to intraperitoneal acetylcholine. Doses that were effective in eliciting abdominal constrictions when injected intraperitoneally (Table 1), were ineffective by the subcutaneous or oral routes in suppressing

TABLE 4

POTENCIES OF SOME ANALGESIC DRUGS AGAINST ABDOMINAL CONSTRICTION RESPONSES INDUCED BY ACETYLCHOLINE AND OTHER CHALLENGE SUBSTANCES

Potencies are expressed as median effective doses (ED50) in mg/kg with 95% fiducial limits, where obtainable, in brackets. The slopes of the antinociceptive dose-response lines are expressed as logits/$\log_{10}$ dose. Challenge substances were injected intraperitoneally 20 min after the analgesic drug had been given subcutaneously. The proportions of mice responding within 2 min were compared with those in control groups not receiving analgesic drug. N.T., not tested ; other details as in Table 1.

	Acetylcholine (3·2 mg/kg)		ATP (4·1 mg/kg)		Bradykinin (0·4 mg/kg)		KCl (2·5% w/v)		Tryptamine (20 mg/kg)	
Analgesic drug	ED50	Slope	ED50	Slope	ED50	Slope	ED50	Slope	ED50	Slope
Codeine	3·89 (3·15–4·86)	1·64	2·93 (1·54–5·56)	4·63	1·58 (0·674–3·70)	3·79	4·97 (2·28–10·8)	1·85	5·16	14·43
Morphine	0·516 (0·459–0·582)	1·61	0·297 (0·204–0·432)	2·05	<0·2	—	0·184 (0·115–0·294)	1·51	0·399 (0· 289–0·548)	2·75
Cyclazocine	0·0790 (0·0649–0·0948)	1·96	<0·0195	—	0·0296 (0·0163–0·0537)	2·27	0·0757 (0·0458–1·24)	1·49	0·0404 (0·0282–0·0583)	2·14
Nalorphine	1·96 (1·04–3·99)	0·37	N.T.	N.T.	0·303	15·09	1·03	0·22	0·769 (0·470–1·26)	0·91
Pentazocine	2·74 (1·75–3·99)	1·48	1·21 (0·588–2·50)	4·16	0·437 (0·192–0·996)	1·66	2·59	1·76	1·96 (1·02–3·77)	1·50
Aspirin Ca	30·4 (25·3–36·7)	1·37	87·2 (69·4–109)	2·47	268 (135–531)	1·05	188 (65·3–543)	0·90	484 (351–666)	2·77
Flufenamate Na	26·1 (20·4–34·0)	2·41	32·8 (22·5–47·7)	3·36	82·0 (53·9–125)	2·41	N.T.	N.T.	182 (41·6–793)	1·15
Meclofenamate Na	9·45 (6·79–13·4)	1·54	6·99 (4·62–10·6)	2·83	18·1 (10·1–32·6)	0·91	N.T.	N.T.	38·6 (31·6–47·2)	3·17
Mefenamate Na	14·8 (10·8–19·9)	1·99	25·2 (17·2–36·8)	3·84	16·4 (10·4–24·7)	2·26	N.T.	N.T.	76·0 (50·3–115)	2·45
Paracetamol	70·9 (45·6–126)	1·50	N.T.	N.T.	>400	—	>400	—	>400	—
Atropine	0·0714 (0·0304–0·118)	0·91	29·4 (18·8–45·8)	2·69	28·4	1·13	4·06 (0·803–20·5)	2·70	3·13	0·64

TABLE 5

POTENCIES OF DRUGS IN ABDOMINAL CONSTRICTION AND DISCO-ORDINATION TESTS

ED50, dose in mg/kg suppressing, in 50% of T.O. strain mice, the abdominal constriction response to intraperitoneal acetylcholine (3·2 mg/kg) ; FD50, dose in mg/kg causing 50% of mice to fall from the surface of a drum rotating at 1 rev/min. Drugs were given 20 min before intraperitoneal challenge. Steroids were given in four daily doses of 100 mg/kg subcutaneously, the last administration being 20 min before challenge. Disco-ordination was tested immediately after the abdominal constriction test was complete (2 min after challenge). P.O., by mouth ; S.C., subcutaneously ; other details as in Tables 1 and 4. Profadol (*CI*-572) is *m*(1-methyl-3-propyl-3-pyrrolidinyl) phenol (Winder, Welford, Wax & Kaump, 1966). Benzydamine is 1-benzyl-3-γ-dimethylaminopropoxy-1H-indazole hydrochloride (Silvestrini, Garau, Pozzatti & Cioli, 1966). Meclofenamic acid (CI-583) is N-(2,6-dichloro-*m*-tolyl) anthranilic acid (Winder, Wax & Welford, 1965). Glifanan is 4-[2′-(β,γ-dihydropropoxy-carbonyl)phenylamino]-7-chloroquinoline (Peterfalvi, Branceni, Azadian-Boulanger, Chifflot & Jequier, 1966). Ketamine (CI-581) is 2-(*o*-chlorophenyl)-2-methylaminocyclohexanone (Chen, Ensor & Bohner, 1966).

Drug			Antinociceptive potency				Disco-ordinative potency				
Class	Individual	Route	No. of mice	ED50	95% fiducial limits	Slope	No. of mice	FD50	95% fiducial limits	Slope	Ratio FD50 ED50
Narcotic and allied	Apomorphine HCl	S.C.	60	0·615	0·266–1·36	1·23	10	>40·0	—	—	>65·0
	Codeine phosphate	S.C.	290	3·89	3·15–4·86	1·64	30	115	82·8–157	6·08	29·6
		P.O.	60	8·44	5·08–13·1	1·96	—	N.T.	—	—	—
	Profadol	S.C.	100	0·860	0·626–1·14	2·17	170	43·0	33·0–63·4	2·62	50·0
		P.O.	70	2·33	1·49–3·63	1·95	—	N.T.	—	—	—
	Dextromethorphan HBr	S.C.	60	4·11	2·33–6·46	1·73	70	75·2	55·8–111	2·81	18·3
	D-propoxyphene HCl	S.C.	30	5·45	2·68–10·4	2·48	99	46·0	31·0–64·4	2·13	8·44
		P.O.	80	5·63	3·87–7·82	2·08	—	N.T.	—	—	—
	Diamorphine HCl	S.C.	30	0·181	0·0842–0·412	1·84	50	9·03	6·20–14·6	2·25	49·9
	Dihydrocodeine H tartrate	S.C.	60	16·7	8·40–28·0	1·68	10	>400	—	—	>24·0
	Methadone HCl	S.C.	30	0·467	0·328–0·645	4·95	80	7·86	4·88–15·6	1·32	16·8
	Morphine sulphate	S.C.	810	0·516	0·459–0·582	1·61	100	81·5	49·9–188	1·15	158
		P.O.	70	3·87	2·15–6·43	1·60	10	> 40·0	—	—	>10·3
	Pethidine HCl	S.C.	60	4·24	3·01–11·5	2·08	30	58·2	40·6–100	3·65	13·7
		P.O.	50	5·81	3·17–9·10	1·96	—	N.T.	—	—	—
	Phenazocine HBr	S.C.	60	0·271	0·221–0·317	5·40	30	*ca.* 4	—	—	*ca.* 15
	Pholcodine tartrate	S.C.	10	> 10	—	—	10	> 10	—	—	—
		P.O.	80	> 40	—	—	—	N.T.	—	—	—
Narcotic antagonist	Cyclazocine HCl	S.C.	310	0·0790	0·0649–0·0948	2·00	180	1·86	1·26–3·66	1·43	23·5
		P.O.	60	2·20	0·704–30·0	0·64	—	N.T.	—	—	—
	Levallorphan tartrate	S.C.	110	4·31	2·48–10·6	0·88	20	> 20·0	—	—	> 4·64
	Nalorphine HBr	S.C.	410	1·96	1·04–3·99	0·37	20	> 40·0	—	—	>20·4
	Pentazocine HCl	S.C.	110	2·74	1·75–3·99	1·48	40	115	84·9–164	5·17	42·0
		P.O.	108	39·4	29·1–55·9	1·96	—	N.T.	—	—	—
Antipyretic	Amidopyrine	S.C.	60	23·9	15·8–34·9	2·06	10	>200	—	—	> 8·36
		P.O.	80	21·6	13·5–32·7	1·53	10	>100	—	—	> 4·63
	Aspirin	P.O.	90	54·6	39·4–74·5	2·05	10	>800	—	—	>14·7
	Aspirin Ca	S.C.	430	30·4	25·2–36·7	1·37	20	>400	—	—	>13·2
		P.O.	60	62·3	45·0–83·0	2·77	10	>200	—	—	> 3·21
	Benzydamine HCl	S.C.	60	7·53	3·68–13·2	1·62	60	> 40	—	—	> 5·31
		P.O.	110	28·9	20·6–41·2	1·70	110	>160	—	—	> 5·54

TABLE 5 cont.

Drug			Antinociceptive potency				Disco-ordinative potency				
Class	Individual	Route	No. of mice	ED50	95% fiducial limits	Slope	No. of mice	FD50	95% fiducial limits	Slope	Ratio FD50 ED50
Antipyretic (*continued*)	Cinchophen	P.O.	30	50·0	30·5–82·0	2·88	10	>100	—	—	> 2·00
	Flufenamate Na	S.C.	110	26·1	20·4–34·0	2·41	120	77·9	61·6–113	2·26	2·98
		P.O.	149	56·4	40·6–75·2	1·53	180	*ca.* 400	—	—	*ca.* 7
	Flufenamic acid	P.O.	180	33·9	21·0–52·5	0·88	10	*ca.* 320	—	—	*ca.* 9
	Glifanan	P.O.	60	97·6	59·6–180	1·60	60	>320	—	—	> 3·28
	Indomethacin	P.O.	150	0·528	0·398–0·694	1·85	10	>160	—	—	>303
	Meclofenamate Na	S.C.	120	9·45	6·79–13·4	1·54	20	> 50·0	—	—	> 5·29
		P.O.	139	11·4	4·87–19·4	0·81	139	>200	—	—	>17·5
	Meclofenamic acid	P.O.	90	26·0	7·88–50·5	0·80	10	>200	—	—	> 7·69
	Mefenamate Na	S.C.	100	14·8	10·8–19·9	1·99	159	57·4	47·0–70·7	2·21	> 3·88
		P.O.	90	76·9	54·2–125	1·56	70	>200	—	—	> 2·6
	Mefenamic acid	P.O.	340	120	86·6–166	0·85	10	*ca.* 800	—	—	*ca.* 7
	Oxycinchophen	P.O.	100	80·0	58·8–124	1·68	20	>160	—	—	> 2·00
	Oxyphenbutazone	P.O.	60	171	122–238	2·74	30	>400	—	—	> 2·34
	Paracetamol	S.C.	70	70·9	45·6–126	1·50	20	>200	—	—	> 2·82
		P.O.	70	124	80·1–190	1·71	20	>400	—	—	> 3·23
	Phenacetin	P.O.	60	105	75·4–149	2·64	10	>400	—	—	> 3·81
	Phenazone	S.C.	30	91·6	43·5–166	2·32	10	>200	—	—	> 2·18
		P.O.	90	84·7	70·9–99·9	4·23	30	>200	—	—	> 2·36
	Phenylbutazone Na	S.C.	30	64·7	39·8–158	2·45	20	<400	—	—	< 6·18
		P.O.	90	62·1	41·7–91·2	1·65	20	>200	—	—	> 3·22
	Salicylate Na	S.C.	80	162	108–230	2·34	10	>800	—	—	> 4·94
		P.O.	70	311	164–544	1·30	20	>800	—	—	> 2·57
Antiacetyl-choline and allied	Atropine sulphate	S.C.	180	0·0714	0·0304–0·118	0·92	60	500	—	0·39	7,000
	Benactyzine HCl	S.C.	80	1·83	0·965–2·99	1·35	100	12·7	7·38–22·5	1·09	6·94
	Decamethonium I	S.C.	40	0·906	0·581–1·27	3·26	30	2·48	1·96–3·12	7·68	2·74
	Hexamethonium Br	S.C.	49	1·05	0·0962–2·98	0·77	30	*ca.* 50	—	—	*ca.* 48
	Hydroxyzine HCl	S.C.	50	10·6	6·68–15·1	3·22	50	22·3	15·3–37·1	2·74	2·10
	Hyoscine HBr	S.C.	50	0·0134	0·00605–0·0296	1·34	10	>400	—	—	>29,900
	Pempidine tartrate	S.C.	40	1·42	0·691–2·80	1·95	10	>200	—	—	>140
Anti-histamine	Chlorpheniramine maleate	S.C.	80	1·54	0·942–2·46	1·44	20	> 24·6	—	—	>16·0
	Diphenhydramine HCl	S.C.	40	5·44	2·73–10·0	2·51	40	52·9	26·9–144	1·86	9·72
	Mepyramine maleate	S.C.	70	6·66	5·62–8·04	6·20	134	55·6	44·3–69·3	2·72	8·35
Anticholin-esterase	Neostigmine methylsulphate	S.C.	40	0·0914	0·0471–0·152	2·15	30	0·0467	0·0201–0·0966	1·89	0·511
	Physostigmine sulphate	S.C.	40	0·0312	0·0139–0·0703	1·44	40	0·0808	0·0343–0·306	1·15	2·59
Sympatho-mimetic and allied	Adrenaline H tartrate	S.C.	213	0·448	0·303–0·596	1·42	30	*ca.* 4	—	—	*ca.* 9
	Amphetamine sulphate	S.C.	50	2·21	1·08–3·91	1·81	88	62·8	49·6–85·8	2·58	28·4
	Cocaine HCl	S.C.	80	2·57	1·46–3·93	1·48	30	15·6	8·67–53·3	1·13	6·07
	Ephedrine HCl	S.C.	30	8·10	3·51–16·1	2·01	30	271	186–477	3·32	33·4
	Imipramine HCl	S.C.	50	10·3	5·85–18·4	1·64	55	162	—	0·59	15·7

TABLE 5 cont.

Drug			Antinociceptive potency				Disco-ordinative potency				
Class	Individual	Route	No. of mice	ED50	95% fiducial limits	Slope	No. of mice	FD50	95% fiducial limits	Slope	Ratio FD50 ED50
Sympathomimetic (*continued*)	Isoprenaline sulphate	S.C.	30	29·4	17·1–394	2·02	10	> 62·5	—	—	> 2·13
	Noradrenaline tartrate	S.C.	70	1·14	0·497–2·04	1·14	20	*ca.* 15	—	—	*ca.* 13
	Tranylcypromine sulphate	S.C.	30	2·31	1·37–3·67	2·90	60	11·2	6·20–25·8	1·28	4·85
CNS depressant	Chlorpromazine HCl	S.C.	70	1·94	1·10–3·53	1·28	70	4·37	3·26–6·90	3·02	2·25
	Ketamine HCl	S.C.	40	7·07	4·51–11·1	2·51	90	26·1	20·1–38·0	2·45	3·69
	Haloperidol	S.C.	50	2·50	1·71–3·65	2·95	10	*ca.* 10	—	—	*ca.* 4
	Methotrimeprazine	S.C.	50	0·234	0·113–0·463	1·77	60	1·75	1·04–2·33	4·07	7·48
	Phencyclidine HCl	S.C.	110	1·62	1·11–2·31	1·59	110	3·06	2·47–3·95	3·50	1·89
	Phenobarbitone Na	S.C.	10	>100	—	—	40	38·2	24·1–62·2	2·36	< 0·382
	Tetrabenazine	S.C.	10	> 40·0	—	—	20	*ca.* 40	—	—	—
	Urethane	S.C.	50	927	612–1,510	2·13	50	753	529–1,000	3·17	0·812
Steroid	Cortisone acetate	S.C.	10	>100	—	—	10	>100	—	—	—
	Dexamethasone	S.C.	10	>100	—	—	10	>100	—	—	—
	Hydrocortisone acetate	S.C.	10	>100	—	—	10	>100	—	—	—
	Paramethasone	S.C.	10	>100	—	—	10	>100	—	—	—
Other	Caffeine citrate	S.C.	30	43·2	19·0–92·7	1·84	70	171	125–244	2·61	3·96
	Ergotamine tartrate	S.C.	140	10·0	5·55–54·3	0·75	30	> 20·0	—	—	> 2·00
	Ergometrine H maleate	S.C.	30	16·1	12·6–25·1	4·90	30	14·1	7·50–26·7	3·65	0·876
	Hydergine	S.C.	10	> 6·00	—	—	10	> 6·00	—	—	—
	Lignocaine HCl	S.C.	39	93·3	53·1–191	2·53	39	55·9	30·7–114	2·03	0·599
	Lysergide	S.C.	30	0·0250	0·0168–0·0372	3·65	30	*ca.* 2·5	—	—	*ca.* 100
	Mephenesin	S.C.	30	68·8	39·9–98·4	3·65	10	*ca.* 200	—	—	*ca.* 3
	Methysergide	S.C.	20	> 16·0	—	—	20	> 16·0	—	—	—
	Papaverine HCl	S.C.	70	90·1	69·9–117	3·13	80	143	102–222	1·83	1·59
	Pentetrazole	S.C.	30	21·8	15·2–29·6	5·16	40	46·9	33·4–67·3	3·90	2·15
	Phenoxybenzamine HCl	S.C.	10	>320	—	—	10	>320	—	—	—
	Phenyl-α-alanine	S.C.	10	>400	—	—	10	>400	—	—	—
	Phenytoin Na	S.C.	10	>400	—	—	10	>400	—	—	—
	Procaine HCl	S.C.	30	35·0	22·4–88·8	2·58	30	>140	—	—	> 4·00
	Pronethalol HCl	S.C.	30	8·05	5·30–11·4	4·01	10	> 20·0	—	—	> 2·48
	Propranolol HCl	S.C.	80	3·86	1·84–8·31	0·89	10	> 40	—	—	>10·4
	Tolazoline HCl	S.C.	50	59·4	36·8–109	1·81	20	>320	—	—	> 5·39
	Urea	S.C.	10	>640	—	—	10	>640	—	—	—

abdominal constrictions induced by acetylcholine. We also tested whether aspirin, calcium aspirin, mefenamate or morphine elicited abdominal constrictions when given intraperitoneally. Neither mefenamate nor morphine induced significantly more responses than did vehicle; aspirin and calcium aspirin induced significantly fewer responses than did vehicle (Table 1).

The rate of onset of the antinociceptive effect of aspirin was investigated in a total of 147 mice by administering graded doses of calcium aspirin intravenously at 2.5, 5, 10 and 20 min before challenge with intraperitoneal acetylcholine. The incidence of abdominal constrictions was significantly reduced at all these times after intravenous treatment. This reduction was maximal at 5 and 10 min.

DISCUSSION

There are several reasons why the abdominal constriction response to intraperitoneal injection of acetylcholine and other substances in Table 1 may be considered nociceptive. First, the response does not differ in appearance, although it may do so in time-course, from that to acetic acid or phenylbenzoquinone, which has usually been considered nociceptive. Second, many of the endogenous substances that elicit this response are known to cause pain or irritation in man (Keele & Armstrong, 1964) and some have been shown to induce nociceptive responses in the dog (Guzman, Braun & Lim, 1962), guinea-pig (Collier & Lee, 1963), or rat (Deffenu, Pegrassi & Lumachi, 1966 ; Blane, 1967). Third, acetylcholine and hypertonic saline caused limping when injected intramuscularly in the mouse. Fourth, the abdominal constriction response to various challenges was suppressed by low doses of morphine and other analgesic drugs.

Table 1 shows that the latency of response is characteristic of the challenge substance. Where a substance has a long latency, as has acetic acid or phenylbenzoquinone, it may be supposed to act indirectly, possibly by liberating an endogenous substance that excites pain endings. An indirect mechanism is less likely where the latency is short, as with acetylcholine, bradykinin and 4% sodium chloride solution (Table 1), each of which may directly excite pain endings. This interpretation of latency is consistent with the findings that 5-hydroxytryptamine and tryptamine each has a much shorter latency than the corresponding amino-acid from which it is derived. The short latency of mustard suggests that it, too, may contain a substance that acts directly on pain endings.

In testing antinociceptive drugs, there are three advantages in using challenge substances that rapidly elicit a response. First, a test takes less time. Second, provided the effect is of short duration, as well as rapid in onset, the exposure of animals to discomfort is curtailed. Third, vehicles induce fewer responses by 2 min than subsequently (Table 1 and Fig. 1).

Emele & Shanaman (1963) have advocated the use of bradykinin as a challenge substance in abdominal constriction tests. They did not, however, describe the rapid onset of responses to bradykinin, and their test, based on responses occurring 20–40 min after challenge, lacks the advantages mentioned. The possibility should be borne in mind (Collier *et al.*, 1964) that substances other than bradykinin, such as chlorbutol and acetic acid, present in the ampoules used by Emele & Shanaman (1963), contributed to the response that they observed.

As Table 4 shows, the immediate responses to intraperitoneal bradykinin can be used for antinociceptive testing. For several reasons, however, we used acetylcholine as the challenge substance in routine tests. First, the effect was rapid in onset (Table 1). Second, it was of short duration (Table 2). Third, a consistently high proportion of mice responded within 2 min to the standard dose (Table 3). Fourth, the standard dose was much less than the acute intraperitoneal LD50. Fifth, challenge could be repeated several times at 20 min intervals. Sixth, the response was easily suppressed by antipyretic drugs and by narcotic antagonists. Seventh, acetylcholine is readily available as a pure substance.

Like acetylcholine, carbachol or nicotine elicits the abdominal constriction response within 2 min of intraperitoneal injection, whereas bethanechol, dimethylphenylpiperazinium or tremorine does not (Table 1). Atropine, hyoscine, hexamethonium or decamethonium antagonizes the response induced by acetylcholine (Table 5). These findings suggest that the mechanism by which acetylcholine elicits the response is not of simple muscarinic or nicotinic type and should be further investigated.

Antipyretic, but not narcotic or narcotic antagonist drugs, more readily suppressed responses induced by acetylcholine than those induced by tryptamine (Table 4). The considerable difference in effectiveness of the antipyretic drugs against these two challenge substances suggests that antipyretic drugs act peripherally in this test.

Acetylcholine had the disadvantage that its noxious effect was also antagonized by atropine (Table 4) and similarly acting drugs (Table 5). In antinociceptive tests, we tried to guard against " false positives," caused by specific antiacetylcholine activity, by also using other challenge substances and by the physostigmine lethality test.

In antinociceptive tests in mice, based on mechanical noxae, such as an artery clip on the base of the tail (Bianchi & Franceschini, 1954), narcotic analgesics, such as morphine, are consistently effective ; but narcotic antagonist and antipyretic drugs show little or no activity (Collier, 1964). In the abdominal constriction test with intraperitoneal acetylcholine, on the contrary, all drugs known to be analgesic in man have proved effective (Table 5). The analgesic potencies of twenty-seven of these drugs are well enough known in man for them to be compared with the potencies against acetylcholine-induced nociception in the mouse (Table 6). From this comparison, very significant ($P<0.001$) correlations in rank order emerge: parenteral, $\rho=0.908$; oral, $\rho=0.821$. In respect of analgesic drugs, therefore, the acetylcholine test predicts very well from mouse to man, and better than does the tail-clip test.

The disadvantage of the acetylcholine test lies rather in the number of " false positives " that it gives. Some of these may be eliminated by the presence of side-effects, including disco-ordination (Table 5). Some " false positive " answers are given by antiacetylcholine and by antihistamine drugs ; but these reduce physostigmine lethality. Sympathomimetic drugs, such as amphetamine and ephedrine, are also positive in the acetylcholine test. Some authors, however, believe that sympathomimetic drugs do have an analgesic effect in man (Ivy, Goetzl, Harris & Burrill, 1944 ; Burrill, Goetzl & Ivy, 1944 ; Harris & Worley, 1957 ; Benassi & Munarini, 1958 ; Randall, 1963 ; Lim, Miller, Guzman, Rodgers, Rogers, Wang, Chao & Shih, 1967). The existence of other " false positive " answers in this test indicates the need for an additional, more selective, test. Because, however, the acetylcholine test placed analgesic drugs in an order comparable with that in man, and because

TABLE 6

COMPARISON OF THE RANK ORDERS OF POTENCY OF ANALGESIC DRUGS IN MAN WITH THOSE OBTAINED FOR SUPPRESSION OF ABDOMINAL CONSTRICTIONS INDUCED BY ACETYLCHOLINE IN THE MOUSE

In man, potencies are based on the doses (in mg of active acid or base/70 kg adult) giving approximately equivalent pain relief. These values are taken from Beaver (1965, 1966) ; *British Pharmaceutical Codex* (1963) ; *Extra Pharmacopoeia* (1967) ; Hook (1966) ; Kast & Collins (1964) ; Keats & Telford (1956) ; Lasagna (1964) ; Lasagna, De Kornfeld & Pearson (1964) ; Light & Fink (1965) ; and Pearson & Lasagna (personal communication). Parenteral treatment in man refers to the subcutaneous or intramuscular route. In the mouse, potencies are based on the antinociceptive ED50 values in Table 5. —, Insufficient data to give a rank order.

	Rank order of potency			
	Parenteral		Oral	
Drug	Man	Mouse	Man	Mouse
Lysergide	1	1	—	—
Cyclazocine	2	2	1	2
Phenazocine	3	5	—	—
Diamorphine	4	3	—	—
Morphine	5	7	2	4
Methadone	6	6	—	—
Nalorphine	7	9	—	—
Methotrimeprazine	8	4	—	—
Profadol	9	8	4	3
Pentazocine	10	10	6	10
Dihydrocodeine	11	14	—	—
Codeine	12	11	5	7
Pethidine	13	12	7	6
D-Propoxyphene	14	13	8	5
Indomethacin	—	—	3	1
Flufenamic acid	—	—	9	9
Phenylbutazone	—	—	10·5	13
Oxyphenbutazone	—	—	10·5	19
Mefenamic acid	—	—	12	17
Amidopyrine	—	—	14·5	8
Aspirin	—	—	14·5	12
Cinchophen	—	—	14·5	11
Phenazone	—	—	14·5	15
Phenacetin	—	—	17	16
Paracetamol	—	—	18	18
Sodium salicylate	—	—	19	20
Oxycinchophen	—	—	20	14
Correlation (ρ)	0·908		0·821	
Significance (P)	<0·001		<0·001	

it yielded no "false negatives," it would seem, with suitable safeguards, to provide a useful, rapid initial screening test for potential analgesic drugs.

In antagonizing nociception induced by bradykinin, antipyretic drugs show very different degrees of effectiveness in different circumstances. Emele & Shanaman (1963) claimed that aspirin more effectively suppressed abdominal constriction responses of mice to bradykinin than those to phenylbenzoquinone. In our hands, however, aspirin, and other antipyretic drugs, though effective, were of lower potency against constrictions induced by bradykinin than against those induced by acetylcholine ; whereas narcotic antagonists were more effective against bradykinin than against acetylcholine (Table 4). Aspirin was ineffective against nociception induced by intradermal bradykinin in the guinea-pig (Collier & Lee, 1963). Lewis (1963) found aspirin 1.2 g by mouth ineffective against pain in man induced by applying bradykinin to an exposed blister base ; but Coffman (1964) found aspirin 6 g just effective against pain induced by intra-arterial

bradykinin. Aspirin and phenylbutazone, but not amidopyrine, inhibited nociceptive responses to bradykinin injected into the splenic artery of the dog (Guzman, Braun, Lim, Potter & Rodgers, 1964). Deffenu *et al.* (1966) found, however, that amidopyrine was about as effective as phenylbutazone and more effective than aspirin in suppressing nociceptive responses to intra-arterial bradykinin in the rat. Blane (1967) could not suppress with mefenamic acid (up to 300 mg/kg intraperitoneally) nociceptive responses to intra-arterial bradykinin in the rat; but we found mefenamate by the subcutaneous route more effective than aspirin against bradykinin-induced constrictions in the mouse.

SUMMARY

1. Of thirty-one substances, other than vehicles, tested for ability to elicit abdominal constriction (writhing) responses when injected intraperitoneally into T.O. strain mice, twenty-six were effective within 10 sec to 30 min. There was a significant incidence of responses within 30 sec of injecting each of twelve substances, including acetylcholine, adenosine triphosphate, bradykinin, histamine, 5-hydroxytryptamine, 2.5% potassium chloride solution, 4% sodium chloride solution or tryptamine. There was a longer delay before the onset of responses to acetic acid, 1.8% calcium chloride solution, chlorbutol, 5-hydroxytryptophan, 2% magnesium sulphate solution, phenylbenzoquinone or tryptophan.

2. To maximally effective intraperitoneal doses of acetylcholine, adenosine triphosphate, bradykinin, potassium chloride or tryptamine, 70% or more mice responded within 2 min. After 2–4 min, the incidence of responses declined.

3. Ten analgesic drugs were tested for ability to lessen the incidence of abdominal constriction responses within 2 min of intraperitoneal injection of at least four of the five challenge substances. Codeine, morphine, cyclazocine, nalorphine, pentazocine, aspirin, flufenamate, meclofenamate and mefenamate were effective to different extents against these challenge substances. Paracetamol was effective only against acetylcholine. All the antipyretic, but none of the narcotic or narcotic antagonist drugs, were more effective against acetylcholine than against tryptamine challenge.

4. Eighty-one drugs were tested as antagonists of abdominal constrictions induced by acetylcholine. All those having analgesic activity in man were effective in the mouse. For twenty-seven analgesic drugs there was a very significant ($P<0.001$) correlation between the rank orders of analgesic potency in man and ability to lessen the incidence of abdominal constrictions induced by acetylcholine in the mouse. Other classes of drugs which inhibited responses to constrictions induced by acetylcholine included sympathomimetic, antiacetylcholine and antihistamine drugs; but supplementary tests eliminated the specific antiacetylcholine and antihistamine drugs used. Hydrocortisone, lignocaine, phenobarbitone, phenytoin, pholcodine and tetrabenazine were among the ineffective drugs.

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