THE ANALGESIC ACTION OF CHLORMEZANONE

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Chlormezanone (2-*p*-chlorophenyltetrahydro-3-methyl-1,3-thiazin-4-one 1,1-dioxide) given to mice by intraperitoneal injection has an analgesic action of its own, and greatly augments the analgesia produced by morphine-type drugs in this species whether the latter drugs are given by the intraperitoneal or intracisternal route. The analgesic effect of intraperitoneal chlormezanone also adds with and prolongs that of paracetamol in mice, is only weakly antagonised by nalorphine and is moderately augmented by ethanol. Chlormezanone has however no analgesic action when given to mice by the intracisternal route. The implications of this last finding are briefly examined experimentally.

ALL the powerful analgesic drugs of the morphine, methadone and pethidine series are drugs of addiction. The weaker antipyretic-analgesic drugs such as acetylsalicylic acid and paracetamol do not suffer from this disadvantage but are ineffective in the relief of severe pain, especially that of visceral origin. Hence any drug capable of augmenting the analgesia caused by these non-addictive drugs appeared to us to merit further study. It was therefore our early observation that chlormezanone could augment the analgesic action of paracetamol in mice that led to this investigation.

EXPERIMENTAL

Detection and Assay of Analgesia

Male white mice, 25–30 g., were distributed at random into groups of either 8 or 10, and separate treatments were assigned to each group. Time-effect curves were constructed for each drug by each route of administration. Individual pain thresholds were recorded in μA needed to elicit a squeak when passed through the body (Lockett and Davis, 1958) before and at intervals after the administration of drugs. Intensity of analgesia was expressed individually as a percentage increase in pain threshold so defined. Assays were made by graded response using 2×2 design and measuring the effect of each drug at the predetermined time of its maximum action.

Drugs. Morphine, methadone and nalorphine were obtained from Burroughs Wellcome and Company Ltd., paracetamol and chlormezanone were gifts from Bayer Products Ltd. These drugs were administered either by intraperitoneal injection in a volume of 0·1 ml., or intracisternally in 0·02 ml. The vehicle used for the intraperitoneal route was either normal saline, in which paracetamol and chlormezanone are ground to fine suspensions, or 1 part of 70 per cent (v/v) ethanol in

9 parts of the saline. All the drugs used were soluble in the latter vehicle which was the only one used for intracisternal injections.

The Metabolism of Chlormezanone by Liver Slices

Slices, 0·7 mm. thick, cut from the livers of freshly killed animals, were weighed and incubated at 38–39° in a nutrient buffered medium containing chlormezanone. The composition of the medium was 0·9 per cent NaCl, 100 parts; 1·15 per cent KCl, 4 parts; 1·2 per cent $CaCl_2$, 3 parts; 3·8 per cent $MgCl_2$, 1 part; 0·1 M phosphate buffer, of pH 7,

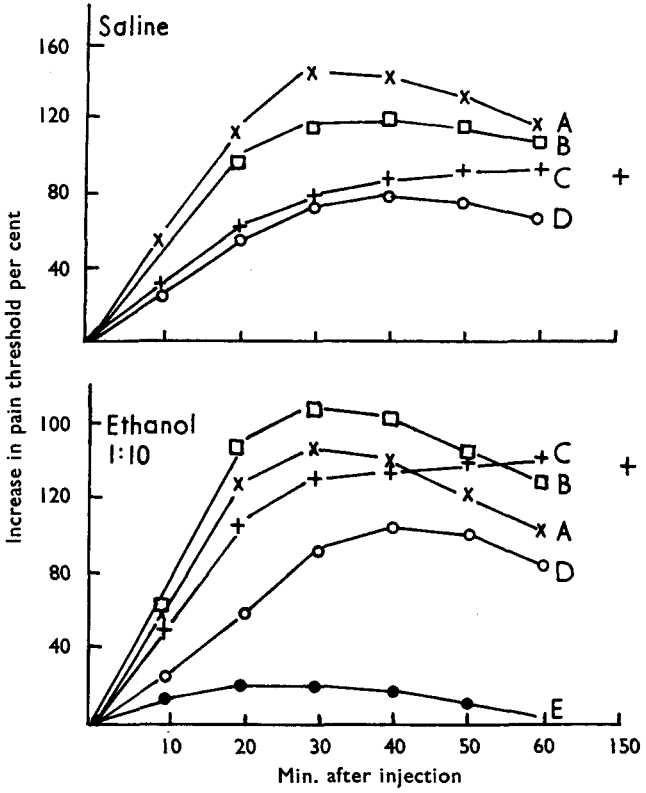

FIG. 1. Comparison of the rates of onset, intensity and duration of analgesia caused by intraperitoneal injections of morphine, pethidine, chlormezanone and paracetamol in mice, ten per group. Vehicles: 0·9 per cent NaCl, and ethanol (70 per cent) 1 in 10 in saline. Drugs: A, morphine HCl, 6 mg./kg.; B, pethidine HCl, 20 mg./kg. (upper), 25 mg./kg. (lower); C, chlormezanone, 50 mg./kg.; D, paracetamol, 300 mg./kg.

20 parts and glucose 1·2 to 2·4 mg./ml., unless otherwise stated. The proteins were coagulated after a 45 min. period of incubation by short immersion of the flasks in a boiling water bath. The flask contents were cooled and centrifuged. Supernatant fluids were examined for analgesic effect by intracisternal injection into mice.

RESULTS

Analgesia Produced by the Intraperitoneal, but not by the Intracisternal, Injection of Chlormezanone in Mice

Comparison was first made of the rate of onset, intensity and duration of analgesia in mice caused by intraperitoneal morphine and pethidine hydrochlorides, chlormezanone and paracetamol, by constructing curves relating the percentage increase in pain threshold to time. Whereas the maximum effect of morphine and pethidine had been reached in 30 min. and of paracetamol in 40–45 min., that of chlormezanone required 60 min. (Fig. 1) and declined little in 2½ hr. The addition of ethanol (70 per cent) (1 in 10) to the saline vehicle slightly increased the rate of onset of analgesia due to all four compounds. It intensified the effects of both paracetamol and chlormezanone, both these drugs being in solution

TABLE I

THE RELATIVE ANALGESIC POTENCIES OF MORPHINE, PARACETAMOL AND CHLORMEZANONE IN MICE. THE FIGURES SHOWN ARE THE MEAN POTENCIES AND STANDARD ERRORS OF THE MEANS, AND THE NUMBER OF ASSAYS WITHIN BRACKETS. THESE ARE RELATED TO PETHIDINE HYDROCHLORIDE (MG./KG. INTRAPERITONEALLY) TO WHICH THE VALUE 1·0 WAS ARBITRARILY ASSIGNED

Drugs	Analgesic potency related to pethidine	
	Drugs suspended in 0·1 ml. aqueous 0·9 per cent solution of NaCl	Drugs dissolved in 0·1 ml. of 1 part 70 per cent ethanol, 9 parts aqueous 0·9 per cent NaCl
Morphine hydrochloride	3·45 ± 0·15 (4)	3·21 ± 0·22 (3)
Paracetamol	0·03 ± 0·002 (3)	0·076 ± 0·002 (3)
Chlormezanone	0·19 ± 0·02 (3)	0·95 ± 0·03 (3)

when injected though they were precipitated in the peritoneal cavity. Next, the relative potencies of morphine and pethidine hydrochlorides, paracetamol and chlormezanone were compared in the presence and absence of this trace of ethanol in a series of 2 × 2 assays in which the maximum effect of each drug was measured and pethidine hydrochloride was the standard of reference. The limits of error (P = 0·95) for the individual assays varied from ±15·2 to ±31·6 per cent. Direct estimates of mean relative potency, followed by the standard error of the mean and the number of assays are shown in Table I. Whereas the trace of ethanol in the second vehicle had itself insignificant effect on pain threshold (Fig. 1) and did not alter the relative potency of morphine and pethidine hydrochlorides, it doubled the analgesic action of paracetamol and increased that of chlormezanone by approximately five times.

In all experiements, the dose effect curves for chlormezanone have tended to be steeper than those for paracetamol, and slightly less steep than the corresponding curves for morphine and pethidine hydrochlorides. On no occasion have these differences been found to be statistically significant.

Chlormezanone failed to influence the pain thresholds of mice when injected intracisternally in doses up to 1 mg./kg. The hydrochlorides of morphine and pethidine had significant action by this route in doses of 4 μg./kg. and 20 μg./kg. respectively.

Augmentation of the Effects of Chlormezanone with Morphine, Pethidine and Paracetamol in Mice

Simultaneous study was made of the effect of a fixed dose of chlormezanone (50 mg./kg.) on the analgesia produced in mice by morphine, pethidine and paracetamol. In this experiment all drug combinations were given in a single intraperitoneal injection (0·1 ml. 0·9 per cent NaCl per mouse). The percentage increases in pain threshold were measured at 10 min. intervals and those found at 30 min. are entered in Table II as means ± their standard errors.

The analgesic actions of chlormezanone and paracetamol little more than added in their intensity (Table II). Synergism was however evident as

TABLE II

AUGMENTATION OF THE ANALGESIC EFFECTS OF MORPHINE, PETHIDINE AND PARACETAMOL IN MICE BY CHLORMEZANONE. ALL COMPOUNDS WERE GIVEN BY THE INTRAPERITONEAL ROUTE; ESTIMATES OF ANALGESIA MADE AFTER 30 MIN. VALUES SHOWN ARE THE MEANS ± STANDARD ERRORS. TEN MICE WERE USED IN EACH DOSE GROUP

Exp. No.	Percentage increases in pain thresholds				Slope of log-dose effect curve for drug I	
	Without chlormezanone		With chlormezanone (50 mg./kg.)		alone	with chlormezanone
	Pethidine hydrochloride		Pethidine hydrochloride			
	20 mg./kg.	40 mg./kg.	20 mg./kg.	40 mg./kg.		
I	55 ± 3·6	81 ± 5·2	87 ± 4·4	162 ± 8·4	87	250
II	62 ± 5·1	98 ± 14·7	108 ± 7·2	202 ± 14·7	120	313
III	64 ± 4·6	106 ± 7·8	124 ± 7·3	208 ± 11·8	140	280
	Morphine hydrochloride		Morphine hydrochloride			
	4 mg./kg.	8 mg./kg.	4 mg./kg.	8 mg./kg.		
I	26 ± 4·1	59 ± 6·3	53 ± 5·6	132 ± 9·4	110	263
II	22 ± 3·6	56 ± 3·8	65 ± 6·2	140 ± 8·7	113	250
III	28 ± 3·2	67 ± 4·4	76 ± 7·4	162 ± 8·8	130	287
	Paracetamol		Paracetamol			
	150 mg./kg.	300 mg./kg.	150 mg./kg.	300 mg./kg.		
I	42 ± 4·4	68 ± 6·4	65 ± 6·6	94 ± 7·5	83	97
II	46 ± 5·2	77 ± 8·3	87 ± 8·2	126 ± 5·8	103	130
III	40 ± 5·5	73 ± 5·8	88 ± 7·0	125 ± 6·6	110	123

Corresponding increases in pain threshold resulting from chlormezanone mg./kg. 50 Exp. I, 21 ± 43. Exp. II, 38 ± 5·1. Exp. III, 44 ± 4·8.

a prolongation of the additive effect (Fig. 2). By contrast, chlormezanone markedly increased the slopes of the log dose per cent effect curves for both morphine and pethidine (Table II), prolonged the duration of their analgesic actions and of observed sedation, but antagonised the respiratory depression which they cause. Intraperitoneal injections of chlormezanone showed similar synergistic action in respect of analgesia and sedation, and again antagonised respiratory depression, when morphine hydrochloride was injected intracisternally. However, intracisternal injection of chlormezanone, even of 1 mg./kg., failed to modify the analgesic, sedative and respiratory effects of morphine given either by the intraperitoneal or by the intracisternal route.

The Effects of Nalorphine on the Analgesic Action of Chlormezanone

The effects of nalorphine on the analgesic and synergistic effects of chlormezanone was now measured in groups of mice. One group received chlormezanone, 50 mg./kg., alone. Other animals received either morphine

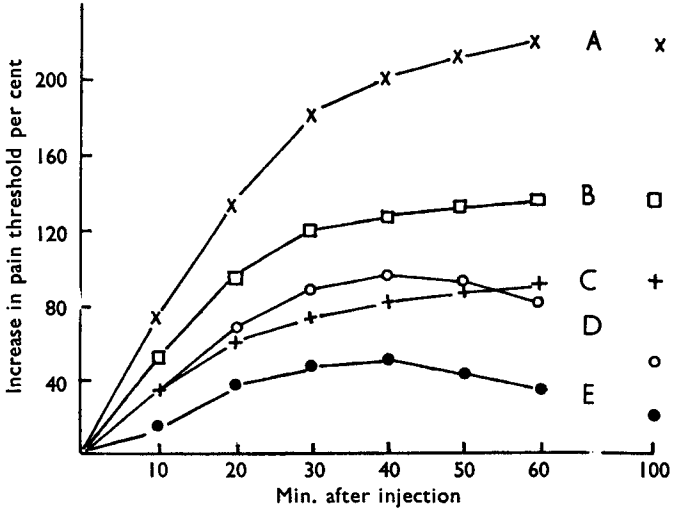

FIG. 2. The additive effects of chlormezanone and paracetamol in mice. A, chlormezanone, 50 mg./kg. and paracetamol 300 mg./kg.; B, chlormezanone, 50 mg./kg. and paracetamol 150 mg./kg.; C, chlormezanone, 50 mg./kg.; D and E, paracetamol 300 and 150 mg./kg. respectively, all given by i.p. injection at zero time.

hydrochloride, 4 mg./kg., or paracetamol, 150 mg./kg. alone, and some were given chlormezanone together with either morphine hydrochloride or paracetamol. Additional groups were treated similarly, but had nalorphine, 4 or 8 mg./kg., as well. All drugs were given in 0·1 ml. 0·9 per cent NaCl by intraperitoneal injection at individual zero times. The percentage increases in pain threshold found 40 min. later have been entered in Table III as means ± their standard errors. The doses of nalorphine used effectively antagonised the analgesic action of morphine and the synergism between morphine and chlormezanone, but caused only

TABLE III

THE ANTAGONISM OF THE ANALGESIC AND SYNERGISTIC ACTIONS OF WIN. 4692 BY NALORPHINE IN MICE. TEN MICE IN EACH DOSE GROUP

Increases per cent in pain threshold 40 min. after intraperitoneal injection						Dose of nalorphine mg./kg.
Without nalorphine			With nalorphine			
Morphine 4 mg./kg.	Chlormezanone 50 mg./kg.	Morphine 4 mg./kg. and chlormezanone 50 mg./kg.	Morphine 4 mg./kg.	Chlormezanone 50mg./ kg.	Morphine 4 mg./kg. and chlormezanone 50 mg./kg.	
39 ± 4·8	34 ± 5·6	87 ± 6·2	16 ± 2·2	38 ± 4·6	41 ± 5·5	4
46 ± 5·3	37 ± 4·7	98 ± 7·1	18 ± 3·6	35 ± 3·7	42 ± 5·8	4
52 ± 6·4	37 ± 5·1	101 ± 6·6	11 ± 3·8	21 ± 5·2	33 ± 5·4	8
43 ± 4·4	36 ± 4·9	91 ± 7·8	9 ± 3·1	26 ± 4·0	28 ± 7·2	8
Paracetamol 150 mg./kg. in place of morphine						
33 ± 5·2	31 ± 5·7	71 ± 6·2	—	—	66 ± 8·4	4
38 ± 3·6	36 ± 4·8	78 ± 7·2	—	—	63 ± 9·6	4

slight to moderate reduction in the analgesic action of chlormezanone and failed to influence the combined effects of this latter drug and paracetamol significantly.

The Production from Chlormezanone by Liver Slices of a Compound or Compounds having Analgesic Action on Intracisternal Injection in Mice

The supernatant fluid obtained after incubation of slices of mouse liver in a nutrient medium containing chlormezanone, 0·1 mg./ml., produced analgesia when 0·02 ml. was injected intracisternally into mice. The

TABLE IV

THE FORMATION OF INTRACISTERNALLY ACTIVE ANALGESIC COMPOUNDS FROM CHLORMEZANONE BY THE LIVER SLICES OF VARIOUS SPECIES DURING ANAEROBIC INCUBATION. THE VALUES SHOWN ARE MEAN INCREASES PER CENT IN PAIN THRESHOLD IN THREE EXPERIMENTS CAUSED BY THE INTRACISTERNAL INJECTION OF 0·02 ML. SUPERNATANT OBTAINED AFTER INCUBATION OF 0·5 G. LIVER SLICES PER 2 ML. MEDIUM CONTAINING CHLORMEZANONE 100μG./ML.

Species	Percentage increase in pain threshold			
	pH 6·6	pH 7·0	pH 7·6	pH 8·0
Mouse	14·2 ± 2·8	36·2 ± 4·5	37·3 ± 4·0	27·2 ± 3·8
Guinea-pig	16·1 ± 2·7	22·5 ± 3·1	24·6 ± 3·2	13·8 ± 1·7
Rat	10·9 ± 2·4	24·1 ± 5·1	26·3 ± 3·9	14·8 ± 3·7
Ox	14·2 ± 4·2	23·8 ± 4·4	24·4 ± 4·7	7·4 ± 2·6
Cat	−1·1 ± 2·2	−0·6 ± 1·7	2·5 ± 3·2	4·1 ± 3·1

TABLE V

THE METABOLITES FROM CHLORMEZANONE, FORMED ANAEROBICALLY BY SLICES OF MOUSE LIVER, WHICH ARE ANALGESIC WHEN INJECTED INTRACISTERNALLY INTO MICE, ADD IN THEIR ACTION WITH MORPHINE BUT ARE ONLY PARTIALLY ANTAGONISED IN THEIR ACTION BY NALORPHINE. THE VALUES SHOWN ARE MEAN VALUES FROM THREE EXPERIMENTS ± THEIR STANDARD ERRORS

Injections made		Increases per cent in pain threshold		
		in the absence of nalorphine		in the presence of nalorphine Mg ions present
Intraperitoneal	Intracisternal	Mg ions present	Mg ions absent	
Morphine	Saline	29·6 ± 3·4	28·3 ± 4·1	6·5 ± 3·1
Morphine	Liver and chlormezanone incubated	50·6 ± 4·6	26·3 ± 5·2	20·2 ± 4·1
Morphine	Liver alone incubated	27·7 ± 5·2	25·8 ± 4·4	7·0 ± 3·6
Saline	Liver and chlormezanone incubated	21·4 ± 3·2	4·2 ± 4·7	15·9 ± 2·1
Morphine	Liver boiled then incubated	25·9 ± 6·1	31·2 ± 4·3	6·8 ± 2·3
Morphine	Liver boiled then incubated with chlormezanone	26·2 ± 4·3	27·4 ± 3·7	7·5 ± 2·8

Morphine, 8 mg./kg. nalorphine 8 mg./kg., by intraperitoneal injection throughout.

results are given in Table IV. Neither the fluid incubated with chlormezanone but without the slices, nor the slices incubated in the fluid without the chlormezanone, nor the complete reaction mixture boiled *before* incubation yielded supernatants which caused analgesia in mice by the intracisternal route. Further work showed that magnesium ions were essential for the production of this analgesic activity, and that the highest yields in the supernatant fluid were reached after 45 min. incubation under nitrogen of 0·5 g. slices in 2 ml. reaction mixture buffered with phosphate to pH 7·0 to 7·6, containing concentrations of chlormezanone within the range 80 to 100 μg./ml.

Slices from the livers of mice, rats, guinea-pigs, rabbits and oxen were all able to produce intracisternally active analgesic compounds from

chlormezanone under these conditions; those from cat livers were not. The volume of supernatant fluid injected intracisternally was always 0·02 ml. per mouse of a solution which had originally contained chlormezanone 100 μg./ml. The weight of active compound injected intracisternally is unlikely therefore to have exceeded 2 μg. per mouse and was probably less. The type of analgesia produced by the metabolite or metabolites of chlormezanone differed from that of the parent compound. Whereas intraperitoneal chlormezanone synergised the analgesic effects of morphine in mice, its metabolic products added in effect with morphine when injected intracisternally (Table V), and were only partially antagonised in their action by nalorphine.

Discussion

That analgesia is produced in mice by intraperitoneal injection but not by the intracisternal injection of chlormezanone indicates that this compound owes its analgesic activity in mice to one or more metabolites produced by tissues other than those of the central nervous system. There is also indication that at least two important metabolites are formed in mice. The first of these has an analgesic action which adds with and prolongs that of paracetamol (Fig. 2 and Table II) and is little antagonised by nalorphine (Table III). The analgesic properties of this hypothetical metabolite produced *in vivo* bear some real resemblance to those of the very active metabolite (Table V) of chlormezanone formed during its anaerobic incubation with slices of liver from many but not from all species (Table IV).

It would seem that a second metabolite is also formed *in vivo*, which potentiates the analgesic action of morphine-type drugs and antagonise the respiratory depression that they cause (Tables II and III). Overall, the evidence would seem to indicate that this second metabolite of chlormezanone is formed outside the nervous system, penetrates into the nervous system, has little or no analgesic action itself (Tables II and III), antagonises morphine's action on respiration, and potentiates its analgesic action.

Few of the many methods used for the assay of analgesia in small laboratory animals can adequately measure the effects of the less powerful analgesic drugs such as paracetamol. Indeed, the method used above, and that in which analgesic action is measured as an antagonism of the writhing movements induced in mice by the intraperitoneal injection of benzoquinone or acetic acid, seem the only methods satisfactory for this purpose. In either case the physiological mechanisms by which pain is registered are complex. Interference with this registration would appear as analgesia. We have no evidence to indicate that the increases in pain threshold we have reported were occasioned by any such interference.

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Reference

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