ANTAGONISM BETWEEN ALKYLATED AND NORCOMPOUNDS OF THE MORPHINE GROUP INJECTED INTRACISTERNALLY INTO MICE

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The analgesia which resulted from the intracisternal injection of mixtures of morphine and normorphine in mice developed more slowly than, and finally equalled but did not exceed that of the more active component of the mixture. Pethidine and morphine antagonised normorphine similarly. However, the analgesia which followed the intracisternal injection of mixtures of pethidine and norpethidine developed as rapidly but was less intense than that due to either single component of the mixtures.

Beckett, Casey and Harper[1] advanced the tentative hypothesis that drugs of the morphine group may require to be dealkylated before they can exert their analgesic effect. The finding by Lockett and Davis[2] that morphine and normorphine had equal analgesic action when injected intracisternally into mice lent some support to this view since the action of normorphine developed a little more rapidly than that of morphine. But, it will be shown by the experiments reported below that normorphine proves less active than morphine when the exposure to ether for intracisternal injection is reduced to less than 30 seconds, although the effect of normorphine on the pain threshold still develops the more quickly. Lockett and Davis also observed that *N*-allylnormorphine antagonised the analgesic actions of intracisternal injections of normorphine and morphine equally and appeared to compete with these two drugs at receptor sites in the central nervous system.

The object of the present work has therefore been to decide, if possible, whether alkylated compounds of the morphine group can produce analgesia by direct combination with receptors, without preliminary dealkylation. This problem was attacked by making comparison in mice of the analgesic actions of alkylated and nor compounds, administered intracisternally, separately and in mixtures. Evidence of initial competition between the alkylated and nor compounds was expected to be followed by evidence of an additive action if the alkylated compounds are active only after dealkylation. Evidence of lasting competition between the alkylated and the norcompounds was expected if both the alkylated and the norcompounds are active analgesic agents. It was a necessary precaution in these experiments to avoid maximum drug effects. Therefore weights of drug were used throughout which produced changes in the pain thresholds less than 50 per cent of those readily induced by these same drugs in mice still showing brisk righting reflexes.

Methods

Male white mice of weight range not exceeding 3 g. in any experiment were divided at random into groups of eight or more. Each mouse was

marked with a dye and the initial pain threshold measured. The intracisternal injection appropriate to the animal's group was made at the individual zero time, and pain thresholds were redetermined 5, 10 and 20 minutes later. The effect of an injection was measured for each mouse as the percentage change in the individual pain threshold. Mean percentage changes were compared by *t* tests; no correction was made for coarse grouping.

Intracisternal injections were made as described by Lockett and Davis[2] except that exposure to ether was reduced to 30 seconds and that neck flexion was minimised. *Pain thresholds* were measured by a modification of the previous method. The earlier tin electrodes were replaced by

TABLE I

ANTAGONISM BETWEEN THE ANALGESIC ACTIONS OF ALKYLATED AND NOR-COMPOUNDS INJECTED INTRACISTERNALLY INTO MICE

Compounds	μg./kg.	No. mice	Percentage increase in pain threshold min. after intracisternal injection		
			5	10	20
Nor–M	15	30	23·7±1·86**	25·2±3·36*	19·6±4·51
M	5	28	15·4±2·49**	26·2±1·96*	22·8±2·03
Nor–M+M	—	31	9·3±2·60	17·1±3·33	19·5±4·69
Nor–M	15	11	14·9±3·58*	15·3±4·40	12·8±5·09
M	2·5	13	6·5±1·86	10·7±2·92*	7·2±2·56**
Nor–M+M	—	12	8·7±1·65	17·3±1·29	17·5±2·51
Nor–M	15	11	15·7±3·19*	17·4±4·25	15·4±5·57
M	1	10	2·1±1·89	1·9±3·07**	0·9±3·03**
Nor–M+M	—	12	6·8±2·06	11·2±1·94	17·0±3·49
Nor–M	15	39	18·4±1·75**	22·2±2·20*	22·7±2·34
P	50	22	4·2±1·74	5·2±2·58*	5·3±3·00**
Nor–M+P	—	39	6·0±1·62	14·4±2·57	22·4±3·31
Nor–P	300	23	12·0±2·33	7·6±1·87	5·2±2·54
P	100	24	14·5±2·13*	14·6±2·01*	11·7±2·45**
Nor–P+P	—	23	7·8±2·07	6·3±2·17	2·8±1·54
Nor–P	300	39	12·1±1·75**	10·5±1·67*	8·7±1·80*
P	50	32	4·7±1·93*	4·8±1·99	4·4±2·39
Nor–P+P	—	38	0·6±0·86	1·5±1·45	2·3±1·73

Mean values are followed by the standard error of the mean. M, P, and Nor- represent morphine, pethidine, and the nor- compounds respectively. When two drugs were given together, the doses used were those immediately above. Significances of differences between the effects of two drugs and single drugs were determined by *t* test and are indicated by asterisks, one, $P = < 0{\cdot}05$, two, $P = < 0{\cdot}001$.

nickel-silver plates and the original very high resistance microammeter was exchanged for one of lower resistance. The modified apparatus had the greater durability and was used as previously described.[2] The values given for the pain thresholds by the modified apparatus were higher than the true values previously recorded because the resistance of the new milliammeter was no longer sufficient to prevent current from passing through it when the key was depressed. Mice with initial 'pain thresholds' outside the range 8–12 micro-amps were rejected. Since background noises could not be entirely eradicated and were found to raise the pain thresholds of mice, control groups of animals were injected with 0·9 per cent NaCl and were examined in parallel with those injected with drug. Corrections were made for changes in the mean pain threshold of control groups when these exceeded 5 per cent; experiments in which this change exceeded 10 per cent were discarded.

RESULTS

Antagonism of the Analgesic Action of Normorphine by Morphine and by Pethidine

In the first experiments, groups of mice received intracisternal injections either of 15 μg. /kg. of normorphine, or of 5 μg./kg. of morphine, or of the two together. The combined results of the three experiments made are shown at the top of Table I. The morphine and the normorphine raised the pain threshold similarly 10 minutes after injection, but the

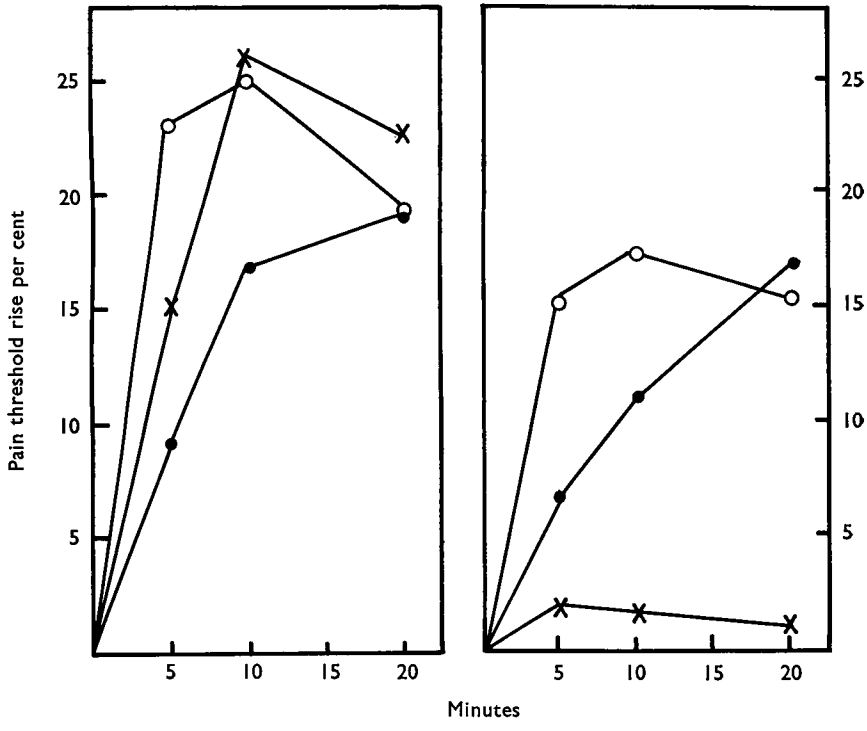

FIG. 1. Antagonism between morphine and normorphine on intracisternal injection into mice. Mean percentage increases in pain threshold are plotted as ordinates against time in minutes as abscissae. See Table I.

O——O 15 μg./kg. of normorphine.
×——× 5 mg./kg. of morphine (L.H. curve).
1 μg./kg. of morphine (R.H. curve).
●——● Combined effect.

effect of the normorphine developed the more rapidly, and waned the sooner. There was highly significant delay in the onset of analgesia when the previous amounts of morphine and normorphine were injected together, for the mean pain thresholds had increased by 23·7, 15·4 and 9·3 per cent respectively, 5 minutes after intracisternal injections of normorphine, morphine and the mixture had been made. The analgesia caused by the two drugs together was still less than that due to either alone

10 minutes after injection, but did not differ from that of either drug in 20 minutes (Fig. 1).

Variation was made in the amount of morphine used in the next experiments. When either 2·5 μg./kg. or 1·0 μg./kg. of morphine was employed and the weight of normorphine remained unchanged, there was again delay in the onset of the analgesic action of the mixture, but then followed a time-effect curve for the mixture not significantly different from that of the normorphine in the absence of morphine. (Table I and Fig. 1.)

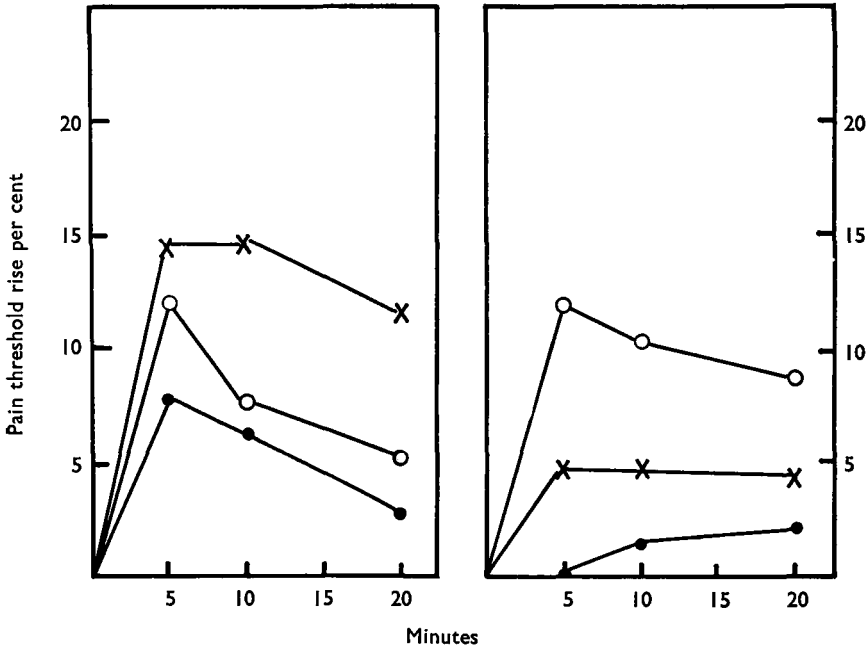

FIG. 2. Antagonism between pethidine and norpethidine on intra-cisternal injection into mice. Mean percentage increases in pain threshold are plotted as ordinates against time in minutes as abscissae. See Table I.

O——O 300 μg./kg. of norpethidine.
×——× 100 μg./kg. of pethidine (L.H. curve).
50 μg./kg. of pethidine (R.H. curve).
●——● Combined effect.

Pethidine 50 μg./kg. also antagonised the onset of analgesia caused by normorphine 15 μg./kg. This effect was evident at 5 minutes, less so at 10 minutes, and had disappeared 20 minutes after injection (Table I).

Antagonism between Pethidine and Norpethidine

Comparable increase in the mean pain threshold was caused by 300 μg./kg. of norpethidine and 100 μg./mg. of pethidine 5 minutes after intracisternal injection into mice (Table I) but the action of the nor-compound disappeared the more rapidly. When these doses of pethidine and norpethidine were given together the analgesia which developed was

delayed in onset, of less intensity than that given by either drug alone, and more like that of norpethidine than pethidine in duration (Table I, Fig. 2).

Pethidine, 50 μg./kg. produced a very small but significant increase in the pain threshold when injected intracisternally into mice. This dose of pethidine completely antagonised the analgesic action of 300 μg./kg. of norpethidine when the two drugs were injected together intracisternally (Table I, Fig. 2).

Discussion

The analgesic actions of intracisternal mixtures of morphine and normorphine developed only slowly to a maximum intensity which equalled that of whichever drug was, by reason of the doses used, the more active component of the mixture. Additive effects from the two components of the mixtures were never seen despite the fact that the changes induced in pain thresholds in the course of these experiments were submaximal and observations were continued for 40 minutes after intracisternal injection. Pethidine and morphine antagonised a more effective dose of normorphine similarly. There was, by contrast no delay in the onset of analgesia when a mixture of pethidine and norpethidine was used and the analgesic action of the mixtures were less than that of either component alone throughout the 40 minutes of observation. There appears therefore to be a difference between the actions of normorphine and norpethidine when these are injected intracisternally. This hypothesis is supported by the fact that the maximum change in pain threshold that can be induced by the intracisternal injection of these two drugs in mice is markedly the greater for normorphine. To postulate that simple competition is occurring between alkylated and nor compounds at common uniform receptor sites in the central nervous system is inadequate to explain these observations. An alternative hypothesis, based on the assumption that the alkylated compounds must be dealkylated before they develop analgesic action is unattractive in the absence of any demonstration of such a dealkylating system in the nervous tissue. The hypothesis could explain the delay in the onset of analgesia after the intracisternal injection of a mixture of morphine and normorphine. There was however no similar delay when a mixture of pethidine and norpethidine was injected. The failure of morphine to antagonise or to sum with normorphine in analgesic effect from the twentieth to the fortieth minute of observation is also in conflict with this hypothesis. It is however conceivable that the concentration of normorphine injected was sufficient to inhibit the proposed dealkylating system If this explanation be accepted, then the rate of dealkylation in the central nervous system must be governed, in large part by the concentration of the dealkylated compound; there may also exist another pathway for the removal of morphine from the sites of analgesic action. This last postulate is made necessary to account for the disappearance of the antagonism between morphine and normorphine by the twentieth minute after intracisternal injection. The prolonged antagonism of norpethidine by pethidine could be explained

similarly if there were no equally effective second pathway for the removal of pethidine from the sites of analgesic action.

It is however evident that neither hypothesis is an adequate explanation of the results of these experiments.

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REFERENCES

1. Beckett, Casey and Harper, *J. Pharm. Pharmacol.*, 1956, **8**, 874.
2. Lockett and Davis, *ibid.*, 1958, **10**, 80.