THE ANALGESIC ACTION OF NORMORPHINE ADMINISTERED INTRACISTERNALLY TO MICE

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The analgesic action of normorphine developed more rapidly than that of morphine and was, weight for weight, as great as that of morphine, and was as effectively antagonised by *N*-allylnormorphine when these drugs were administered intracisternally to mice. By this route, more than one molecule of *N*-allylnormorphine was required to antagonise the action of one molecule of analgesic drug.

SPECIFIC receptors, of predicted chemical and physical character, were postulated for the analgesic actions of drugs of the morphine, methadone, and pethidine series[1]. Later, Beckett, Casy, and Harper[2] brought forward a theory concerning the mode of action of morphine-type drugs which may be briefly summarised as follows. All *N*-alkylated drugs which are, by reason of their chemical structure and spatial configuration, a fit for these specific "analgesic receptor sites", become adsorbed on these sites. The formation of this drug-receptor complex does not itself produce analgesia. Analgesia occurs only if an oxidative dealkylation takes place on the receptor surface, with the production of the dealkylated (nor-) drug. Analgesia is the product of a further interaction in which the dealkylated compound takes part.

The object of the investigation has been to test two predictions which can be made from this theory. First, the analgesic action of morphine has been attributed solely to that of the normorphine formed from it by demethylation. If this is true, the analgesic action of normorphine should prove as great as that of morphine and be the more rapid in onset. The analgesic actions of these two drugs have therefore been compared. Secondly, comparison has been made of the antagonism by *N*-allylnormorphine (nalorphine) of the analgesias produced by morphine and by normorphine. According to the theory nalorphine should be attracted to the specific receptor sites for dealkylation, for its own very weak analgesic action[3,4] should be attributed to an exceedingly slow formation of normorphine from nalorphine by dealkylation. Accordingly, the action of nalorphine as an antagonist of morphine is most easily explained as one of competition between these two drugs for the specific receptors for dealkylation. If this is the true explanation of the action of nalorphine, then nalorphine should prove an antagonist of morphine but not of normorphine. This point was therefore examined.

Mice were used for these investigations, and drugs were administered by the intracisternal route to minimise the effects of any difference in the rates of destruction of morphine and normorphine in the animal body.

METHODS

Male white mice, of weight range not exceeding 2 g., were distributed at random into groups of ten.

Drugs. Morphine and nalorphine (British Drug Houses Ltd.) and normorphine (by courtesy of Beckett, Casy, and Harper) were obtained and used as hydrochlorides, and were dissolved in 0·9 per cent w/v aqueous sodium chloride for injection.

Intracisternal injections. Gauge 26 intradermal needles were bent away from the bevel one quarter of an inch from the tip through an angle of approximately 20°. The head of each mouse was bent well forward, under ether anaesthesia, and the tip of the needle was passed through the intact skin, muscles, and ligaments in the midline at the back of the neck, so that the point slipped in between the occiput and the atlas vertebra. The bent part of the needle was then pushed forward, keeping it in close contact with the internal surface of the occiput, for its whole length. An injection of 0·02 ml. was made and the needle was withdrawn. Normal pain thresholds were restored in less than five minutes after the injection of 0·9 per cent sodium chloride. The distribution of such an injection of 0·1 per cent Evans Blue was observed in ten mice. Each was injected, then immediately killed by deepening the anaesthesia. The vault of the skull was at once cut away with a very sharp scalpel. The dye had covered the base of the brain and had passed into the third and fourth ventricles of all the mice. It had outlined the lateral ventricals in eight out of the ten. It had extended over the surface of the cerebral hemispheres very considerably, but to a variable extent. It had leaked back into the muscles of the neck appreciably in two cases. *Intravenous injections* were made into tail veins.

Analgesia was measured by means of a much modified Singh Grewel apparatus and procedure. The electrode system used consisted of a saline covered tinned metal plate insulated from a saline filled tinned metal trough. The mouse stood on the plate with its tail passing through a slot in an upright, which separated the electrodes, into the trough. Alternating current was supplied to the electrodes from the 12 volt tapping of a bell transformer whenever a key was depressed. The voltage was varied by means of a potentiometer, and the current flowing through the mouse on depression of the key was measured with an A.C. microammeter placed in the electrode circuit. *Pain thresholds* were measured as follows:—The key was tapped smartly four times at a current flow of 6 μ amps. This was repeated, increasing the current flow through the electrodes in increments of either 1 or 2 μ amps after each trial, until the mouse squeaked. The current value which elicited the squeak was recorded as the pain threshold. Mean pain thresholds were compared by "*t*" tests; no correction was made for coarse grouping.

Results

A Comparison of the Analgesic Actions of Morphine and Normorphine

Both the intensity and the rate of onset of the analgesic action of normorphine have been compared with those of morphine. In the first six experiments both drugs were given by intracisternal injection. In three, two groups of fifteen mice were used and pain thresholds were

determined for each mouse before, and 10, 20, and 30 minutes after a drug was given. In the other three experiments there were twelve mice in each group, and pain thresholds were measured before and 5 minutes after the drug was injected. Mice of one group were injected with morphine hydrochloride, 30 μg./kg. intracisternally, and those of the other group with normorphine hydrochloride, 30 μg./kg. similarly. The results of these experiments have been summarised in Table I. Five

TABLE I

A COMPARISON OF THE EFFECTS OF MORPHINE AND NORMORPHINE HYDROCHLORIDES, INJECTED INTRACISTERNALLY IN A DOSE OF 30 μg./kg., ON THE PAIN THRESHOLD OF MICE

Min. after injection (intracisternal)	*Pain threshold in μ amps. $\pm$ S.E. (No. of mice)	
	Morphine	Normorphine
0	10·36 $\pm$ 0·20 (45)	10·47 $\pm$ 0·19 (45)
10	12·80 $\pm$ 0·41 (45)	12·76 $\pm$ 0·29 (45)
20	13·69 $\pm$ 0·28 (45)	12·58 $\pm$ 0·31 (45)
30	14·45 $\pm$ 0·31 (45)	12·53 $\pm$ 0·35 (45)
0	10·01 $\pm$ 0·34 (45)	10·31 $\pm$ 0·27 (45)
5	12·22 $\pm$ 0·26 (45)	13·89 $\pm$ 0·29 (45)

* Pain thresholds in μ amps are expressed as means, plus or minus the standard error of the mean, followed by the number of mice within brackets.

minutes after intracisternal injection, normorphine proved more effective than morphine in raising the pain thresholds of mice ("t" calc. = 3·0, $n = 66$). Thereafter, the action of normorphine slowly decreased and that of morphine gradually increased. There was, therefore, no difference in the intensity of the analgesia caused by equal weights of these drugs

TABLE II

A COMPARISON OF THE EFFECTS OF MORPHINE AND NORMORPHINE HYDROCHLORIDES, INJECTED INTRAVENOUSLY, ON THE PAIN THRESHOLD OF MICE

Hydrochloride injected, mg./kg.		Min. after injection (intravenous)	*Pain threshold in μ amps. $\pm$ S.E. (No. of mice)	
Morphine	Normorphine		Morphine	Normorphine
10	50	0	10·44 $\pm$ 0·32 (20)	10·61 $\pm$ 0·42 (20)
		10	18·61 $\pm$ 0·47 (20)	15·67 $\pm$ 0·53 (20)
		20	19·42 $\pm$ 0·51 (20)	14·68 $\pm$ 0·67 (20)
10	100	0	10·14 $\pm$ 0·33 (10)	10·31 $\pm$ 0·36 (10)
		10	17·97 $\pm$ 0·55 (10)	20·78 $\pm$ 0·82 (10)
		20	19·78 $\pm$ 0·63 (10)	19·13 $\pm$ 0·64 (10)

* Pain thresholds in μ amps are expressed as means, plus or minus the standard error of the mean, followed by the number of mice within brackets.

10 minutes after their intracisternal injection, but the action of morphine became significantly the greater after 20 minutes ("t" calc. = 2·53, $n = 84$) and remained so.

Very different results were obtained when these two drugs were given by intravenous injection instead of intracisternally. Two experiments were made in which there were ten mice in each group, and pain thresholds

were determined for each mouse before, and 10 and 20 minutes after the intravenous injection of either morphine, or normorphine, hydrochloride. The combined results of these experiments are shown in Table II. Whereas the analgesic action of normorphine reached maximum intensity in 10 minutes, that of morphine continued to develop for 20 minutes. However, the analgesic potency of normorphine was approximately one tenth of that of morphine when the drugs were given intravenously.

The Antagonism of the Analgesic Actions of Normorphine and of Morphine by Nalorphine

The antagonism of the analgesic actions of morphine and of normorphine by nalorphine has been studied in mice using a single intracisternal injection for the administration of drugs. Each injection therefore contained either an activating drug alone, or both an activating drug and

TABLE III

MEASUREMENT OF THE ANTAGONISM OF THE ANALGESIC ACTIONS OF MORPHINE AND NORMORPHINE BY NALORPHINE IN MICE

Content of intracisternal injection, μg./kg.			Mean pain threshold in μ amps. ± S.E. (No. of mice)	
Morphine	Normorphine	Nalorphine	Before injection	20 min. after injection
10	—	—	11·0 ± 0·33 (10)	14·0 ± 0·30 (10)
10	—	10	10·8 ± 0·37 (10)	12·4 ± 0·65 (10)
—	10	—	10·8 ± 0·44 (10)	14·2 ± 0·61 (10)
—	10	10	11·2 ± 0·33 (10)	12·4 ± 0·96 (10)

the inhibitor drug, and was of standard volume, 0·02 ml. Since two activator and one inhibitor drug were studied, four groups of mice were used in each experiment. Pain thresholds were determined for the ten mice in each group before and after the intracisternal injection. The post-injection interval before the pain thresholds were measured for the second time was 20 minutes in the first six experiments. In these experiments, therefore, a constant contact period was allowed between the inhibitor drug and the receptors, but the effect of the inhibitor was measured during the development of the action of morphine and the regression of that of normorphine. The next five experiments differed from the first six in that the effects of nalorphine were determined at the time of the maximum action of the analgesic drug. The second measurement of the pain threshold was therefore made 5 minutes after the intracisternal injection of normorphine, and 30 minutes after that of morphine. The results of a single experiment of the first series are shown in Table III. These results were typical of all the experiments in which the antagonism of the actions of morphine and normorphine by nalorphine were studied. First, there was no significant difference between the initial mean pain thresholds of the different groups of mice. Secondly, these pain thresholds were significantly increased by all four treatments. Thirdly, the increase in the pain threshold which followed the intracisternal injection of the analgesic drug alone significantly exceeded that which resulted from the simultaneous injection of both the analgesic drug and nalorphine.

The percentage increases in pain threshold caused by morphine and by normorphine in the presence and absence of inhibitor have been entered, for each experiment, in Table IV. The first six experiments listed are those in which pain thresholds were measured 20 minutes after the drugs were injected intracisternally. There was great similarity in the antagonism of the analgesic actions of morphine and of normorphine in these experiments. In the next five experiments, the effects of normorphine,

TABLE IV

A COMPARISON OF THE ANTAGONISM OF THE ANALGESIC ACTIONS OF MORPHINE AND NORMORPHINE BY NALORPHINE, BY THE INTRACISTERNAL INJECTION OF THESE DRUGS INTO MICE. SEE TEXT FOR EXPLANATION

Injected drugs μg./kg.		Increase per cent in pain threshold caused by drugs			
Analgesic	Antagonist	Morphine	Morphine and nalorphine	Normorphine	Normorphine and nalorphine
10	10	27·3	14·8	31·5	10·7
		23·1	10·7	25·5	10·9
50	25	64·0	44·0	73·0	49·0
		68·2	51·6	67·6	49·6
100	500	88·7	9·8	120·5	18·1
		91·9	15·0	118·9	15·1
50	25	46·0	33·3	46·9	30·5
		42·7	26·8	47·0	32·9
50	100	53·9	15·6	55·0	24·8
		45·1	19·1	52·9	20·8
		42·7	16·7	47·1	21·9

in the presence and absence of nalorphine, were measured 5 minutes, and those of morphine 30 minutes after intracisternal injection. The fact that nalorphine again proved equally effective an antagonist of normorphine as of morphine under these circumstances indicated that the full effect of nalorphine had developed within 5 minutes of its intracisternal injection. The overall results in this Table show that at least one molecule of nalorphine is required to antagonise one molecule of either morphine or normorphine when these drugs are administered by intracisternal injection.

DISCUSSION

The analgesic action of normorphine developed more rapidly than that of morphine and was, weight for weight, as great as that of morphine when these drugs were administered by intracisternal injection. These observations are compatible with the hypothesis that *N*-alkylated drugs of the morphine type must undergo dealkylation before they can produce analgesia[2]. A decrease in the relative analgesic strength of normorphine on intravenous injection is probably to be attributed to the more rapid destruction of this secondary amine in the animal body, and to its less efficient penetration from the blood to the central nervous tissue, by comparison with morphine.

Nalorphine proved as effective an antagonist of normorphine as of morphine, and more than one molecule of the inhibitor was required completely to antagonise the action of one molecule of activator drug, when all drugs were administered by intracisternal injection. These

facts are more difficult to reconcile with the hypothesis of Beckett, Casy and Harper[2]. Previous estimates of the potency of nalorphine as an antagonist of the analgesic action of morphine have been made by the systemic route of administration. Under these conditions nalorphine antagonised many times its molecular equivalent of morphine[4].

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