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THE EVALUATION OF ANALGESIC POTENCY OF DRUGS USING THERMAL STIMULATION IN THE RAT

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Numerous methods have been devised for the application of noxious stimuli to a variety of experimental animals in an endeavour to find a satisfactory method of estimating the analgesic potency of drugs. The stimulus applied produces a protective response of some kind which is interpreted as a pain response, and the effect upon it of the drug under investigation may then be studied. One may doubt the validity of interpreting the human experience of pain in terms of the objective response of an animal. The results of recent years, however, do appear to show some parallel between relative potency as estimated in animals and clinical experience in the use of drugs of the morphine group and their synthetic " analogues."

Of recent years the use of thermal stimuli in the form of radiant heat has come into general use for the investigation of analgesic phenomena in man and animals. The method devised by Hardy, Wolff, and Goodell (1940) has been much used on man by a variety of investigators; in this country attempts to use this technique have not met with success (Dodds, Lawson, Simpson, and Williams, 1945 ; Thorp, 1946). Application of similar techniques to the rat, however, have been uniformly satisfactory. This anomaly formed the basis of the present investigations, which were undertaken in an attempt to find an explanation of the discrepancy.

When radiant heat is used there are two methods of grading the stimulus :

(1) Exposure is permitted for a fixed time interval and the intensity of the radiation varied to produce reaction just within this time. Any increase in the intensity of radiation required to produce the response under the influence of a drug is interpreted as depression of the pain sense (Thorp, 1946).

(2) The time for reaction to a fixed stimulus is measured and prolongation of this beyond the normal is taken as an indication of analgesia (D'Amour and Smith, 1941 ; Davies, Raventos, and Walpole, 1946). Such experiments are less well controlled, since the intensity of the radiant heat stimulus is not necessarily increasing proportionately with time.

No comparison can be made, by radiant heat techniques, of the normal thermal thresholds in man and the rat or of the threshold elevating effects of analgesic drugs in the two species. In the present study it was decided that temperature measurement would be a more reliable index of the response to thermal stimulation ; a direct comparison should then be possible between the interpretation of a pain response by the human subject and the objective response of the rat.

METHODS

The apparatus consists of a heating element shown in the accompanying photographs (Figs. 1 and 2). A strip of uniform oxidized " Nichrom " tape (0.25 inch width $\times$ 0.006 inch thickness) is shaped over one area so as to form an almost complete circle which is to enclose part of the rat tail. This loop lies transversely across a channel in a small board along which the tail lies. The free ends of the resistance tape are secured to the board—one permanently (A, Fig. 1), the other slotted and held down by two pieces of plastic (B and C). Lateral pressure on the extremity (F) enables the circumference of the heater loop to be varied at will, so as to fit tails of varying size. The screw (D) passes through the slotted resistance tape and enables this to be fixed in position. The resistance tape has sufficient flexibility for this purpose, whilst its thermal capacity is low so that further heating of the tail when the current

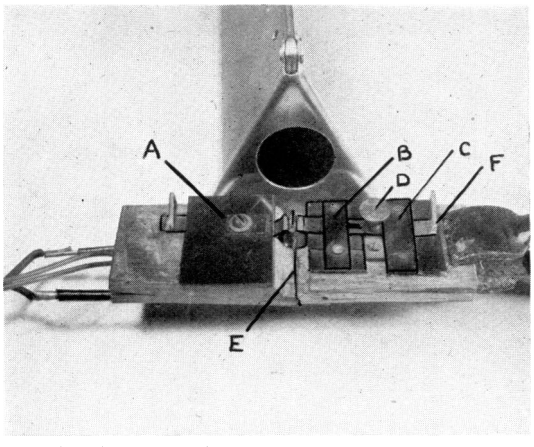

FIG. 1.—Apparatus for the application of a measured thermal stimulus to a rat tail.

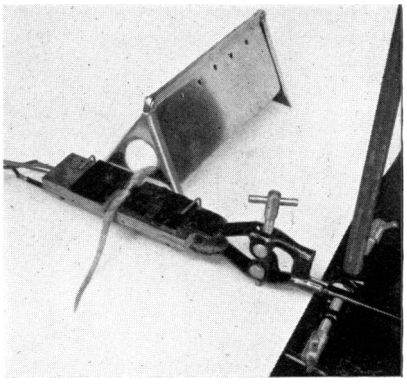

FIG. 2.—Apparatus for thermal stimulation in use; note the rat in specially designed holder.

is discontinued does not occur. Its resistance permits rapid heating with an alternating current of 5.5–6.5 amp. at 8V. Firm but not tight contact between heater and tail is essential. The temperature at which the tail reaction occurs is measured by a thermocouple of copper-constantan (E) lying transversely across the heater loop, the wires running along the tail groove. The couple must be sensitive and of small thermal capacity, otherwise low temperature readings are obtained. The wires used were of 32 gauge (0.011 inch); the ends were beaten out flat to form very thin plates which were then joined with a minimal amount of solder. A cold junction in ice was included in the circuit and readings made on a sensitive galvanometer.

When the heater is used in an experiment, it is not possible to assess the accuracy with which the temperature measurement is made. In view, however, of the results of numerous experiments carried out in man with a similar device, it is believed that the temperature readings approximate to the actual skin contact temperature applied. At first considerable difficulty was experienced in the matter of holding animals in position during heating, for it is essential that they remain quiet. Ultimately a holder was devised which has proved satisfactory (Figs. 1 and 2). In this holder ordinary Wistar strain rats may be used without previous training; the shape of the holder is such that the animal does not possess the sense of instability which cylindrical holders give and is unable to rotate along a longitudinal axis, which is a great trouble with tubular holders.

The only preliminary treatment required by the animals for the technique consists in shaving the tails over about one inch of length towards the base so as to ensure close contact between the epidermis and heater; otherwise unsatisfactory readings are obtained. This procedure is carried out the previous day so that the hypersensitivity induced settles down before an experiment is carried out.

In the weight group used (120–150 g.) the normal temperature of reaction falls within quite a narrow range (38°–40° C.) for most animals. It tends to rise somewhat in heavier animals, owing possibly to the increased thickness of the epidermis. The normal response consists of a sudden sharp twitch of the tail, which often disengages it from the grip of the heater; should any attempt be made to continue the stimulus, a struggle follows, which suggests that a pain threshold corresponds with the initial movement.

When the temperature of reaction rises under the influence of an analgesic drug, damage to the skin may occur, if heating is uncontrolled, depending upon the maximum temperature applied and the period of heating. In practice the duration of the stimulus is kept round about 10–15 seconds by means of a variable choke and the maximum temperature applied is 48° C. If no twitch occurs under these circumstances " analgesia " is assessed as complete. Response rarely occurs if this temperature is exceeded and damage is likely.

TABLE I

Scheme of testing for analgesic potency showing some results (0–100% response) for ketobemidone. For practical purposes it is unnecessary to convert the galvanometer readings to degrees centigrade. The reading " 75− " indicates no reaction of the rat tail at the maximum permitted temperature (48°) and is assessed as " complete analgesia." The average normal reaction is in the range 38–40°. " 75± " indicates uncertainty over the response and is always followed by further readings. Note the submaximal elevations shown by animals 3 and 5 (0.5 mg./kg.)

No. of rat	Weight (g.)	Dose		Galvanometer readings				
		mg./kg.	Volume ml.	Normal	1 min.	5 min.	10 min.	15 min.
1	129	1	0.13	60 65	75− 75−	75− 75−	75− 75−	75− 75−
2	158	1	0.16	59 58	75− 75−	75− 75−	75− 75−	75− 75−
3	157	1	0.16	59 59	75− 75−	75− 75−	75− 75−	75− 75−
4	140	1	0.14	63 62	75− 75−	75− 75−	75− 75−	75− 75−
5	152	1	0.15	61 64 59	75− 75−	75− 75−	75− 75−	75− 75−
1	186	0.75	0.19	63 64	70 71	75± 75−	75− 75−	75− 75−
2	176	0.75	0.18	64 64	75− 75−	75− 75−	75± 75− 70	75− 75−
3	180	0.75	0.18	67 65	75± 75− 75−	75± 75−	75− 75−	75− 75−
4	176	0.75	0.18	64 65	75− 75−	75± 73 70	75± 69 63	75± 69 69
5	189	0.75	0.19	63 69 68	75− 75−	75− 75−	75− 75−	75− 75−
1	134	0.5	0.13	60	64 65	74 66 67	68 66	58 59
2	138	0.5	0.14	65 56 63 60	65 65	67 68	65 65	64 65
3	154	0.5	0.15	58 58	71 73	71 72	71 75	72 73
4	133	0.5	0.13	57 60	60 61	61 64	59 60	58 61
5	130	0.5	0.13	56 57	73 74	71 72	64 67 69	64 65

In the present experiments the rats used were of an inbred Wistar strain of American origin. For each dose level, groups of 5 to 10 rats of either sex selected at random were used; each animal was used for one experiment only. They were introduced into holders, allowed to settle down for a few minutes, and control threshold levels taken to ensure that they reacted normally. The tail was next immersed in water at 45° C. for 90 seconds to dilate the tail veins and the drug under test injected in a dose volume of 0.1 ml./100 g. in about 2–3 sec. Three successive estimates of the end point were made at intervals of 1, 5, 10, and 15 min. after the injection, indiscriminate sites on the shaved area of the tail being used. By suitable timing a group of five animals can be dealt with in about thirty minutes. " Analgesia " rarely develops later than five minutes after intravenous administration of a drug. Reference to the specimen experimental Table I will clarify the procedure adopted.

RESULTS

Application of a thermal stimulus to the tail as described forms a reliable method of eliciting a motor response. Of a number of factors which might influence the normal temperature of reaction, only the initial temperature of the tail, which varies widely with room temperature, and the duration of the stimulus need be considered. A low initial temperature produces an earlier reaction temperature, so that successive readings from higher initial temperatures result in a stepwise rise of threshold figures. A relatively constant level is soon reached, however, and it is then unrelated to the initial temperature if this is above 30° C. These effects are best observed in animals under the influence of a hypnotic, since normal animals quickly grow restive with repeated stimuli (Fig. 3). The reaction temperature is independent of the duration

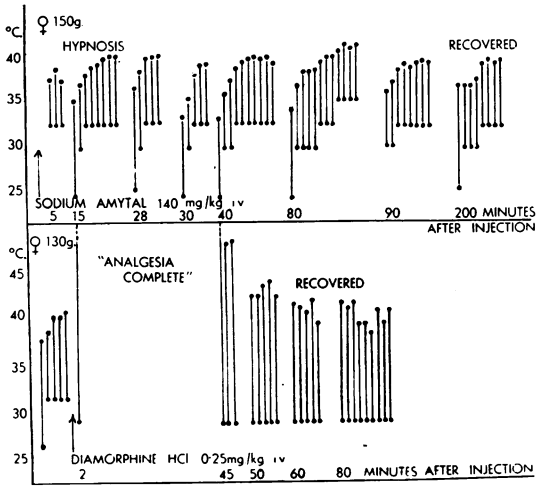

FIG. 3.—*Upper graph.* The temperature of reaction of the rat tail to thermal stimulation under hypnosis. Sodium amytal, 140 mg./kg., i.v. *Lower graph.* The temperature of reaction after intravenous diamorphine (0.25 mg./kg.) for comparison. Successive stimuli were applied at intervals of about one minute, commencing at the times indicated. Each vertical line represents the temperature range covered by the stimulus.

of the stimulus. By varying the heater current the same threshold is obtained irrespective of a long or short period of heating ; this holds also in man.

The effect on the reaction temperature of a number of analgesic drugs has been studied. After a suitable intravenous dose the " analgesic " effect develops so rapidly that readings after one minute almost invariably show the maximum effect ; the fall to normal levels is also quite a rapid process. With smaller doses threshold

increments varying from animal to animal are obtained. The rise in threshold may be very transient and appear only in tests carried out after one or five minutes. Davies, Raventos, and Walpole (1946) took readings fifteen minutes after intravenous injections. " Analgesic " effects of shorter duration, easily detectable by the present method, would not be recorded by their technique. Since their method is based upon measuring the reaction time to an uncontrolled stimulus, it is possible that the marked fall in tail temperature which occurs after such drugs may be responsible in part for prolonging the reaction time.

The dose-response curves for six drugs are shown in Fig. 4. A comparison of the potencies of these substances on the basis of " intensity " of effect has been made by estimating the dose required to produce the maximum response in 50 per cent of a group of animals (AD50) at any of the specified times of testing (Table II). Two successive elevations of the threshold to maximal values are stipulated in order

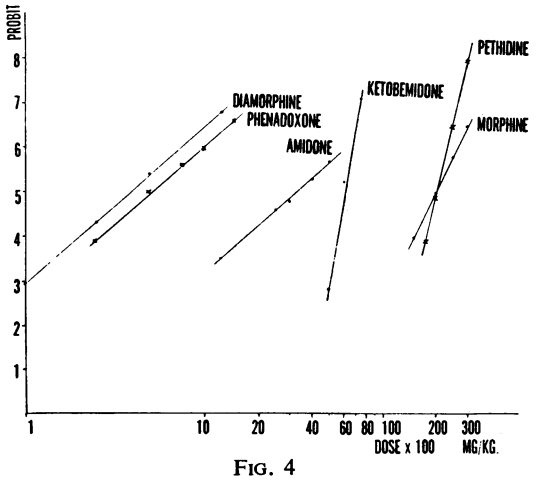

FIG. 4

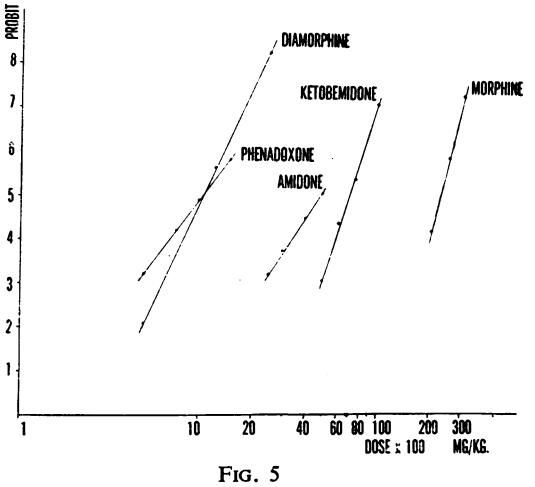

FIG. 5

FIG. 4.—The relationship between dose of drug and intensity of " analgesic " effect expressed as probit-response lines. Each point on the curves represents the frequency of occurrence of the maximal elevation of threshold as defined, in the 15 minutes following intravenous administration of the drug.

FIG. 5.—Probit-response lines for six analgesic drugs expressing the relation between dose and the number of animals showing a continued state of maximum " analgesia " for at least 15 minutes after intravenous administration of the drug.

to avoid occasional false readings which may occur. The " duration " of effect has also been assessed for the same dose range in terms of the amount of drug which will maintain the defined maximum level of " analgesia " for at least 15 min. in 50 per cent of a group of animals. The probit-response curves on this basis are presented in Fig. 5, and the AD50 values in Table III.

The intravenous toxicities of some of these drugs (Table IV) were determined in groups of five animals for each dose level (in a volume dose of 0.1 ml. per 100 g. weight). The mortality was recorded in deaths occurring within 24 hours.

Discussion

The intravenous analgesic potencies of morphine and pethidine according to Davies, Raventos, and Walpole (1946) are 2.2 and 4.6 mg./kg. respectively, as compared with 2.0 and 2.2. mg./kg. using the technique described in this paper. Since the above authors measured their analgesic effect 15 min. after injection and the present paper illustrates the brief duration of analgesic effect after intravenous pethidine (Table III), it is not surprising that Davies *et al.* found a higher dose of pethidine was required. Thorp (1946) and Basil, Edge, and Somers (1950), also using a radiant heat stimulus, administered their drugs subcutaneously so that rather larger doses of morphine were required to produce the required analgesic effect compared with the intravenous route (see below). From the present series of tests the interesting observation appears that diamorphine and phenadoxone are some fifty and forty times as potent respectively as morphine. These are much greater relative effects than previous workers have found, as the following figures show :

	Activity (morphine=1)			A.D.50 Morphine
	Phenadoxone	Diamorphine	Amidone	
Thorp (1946)	—	7	1.83	3.5
Basil *et al.* (1950)	3.8	—	1.3	3.67
Jackson	40	50	6.5	2.0

This enhancement of potency is clearly related to the route of administration, since the efficiency of morphine is not much altered by a change from subcutaneous to the intravenous route. It may be that phenadoxone and diamorphine are rapidly eliminated or destroyed so that the slower absorption from a subcutaneous site results in a relatively greater loss of potency compared with intravenous administration. The dose response curves for pethidine and ketobemidone are very steep and parallel, which is interesting in view of their similar chemical structure. A two-fold increase in dose is approximately adequate to cover the range of zero to 100 per cent " analgesia " for these two substances and morphine, whereas diamorphine and phenadoxone require about a tenfold increase. Amidone appears to fall in an intermediate range between these two groups. The ability of these drugs to maintain the maximum " analgesic " effect for at least 15 min. after injection is illustrated in

TABLE II

The intravenous potency of analgesic drugs in rats to a contact heat stimulus, based on the intensity of analgesic effect

Drug	AD50 (intensity) mg./kg.	Limits (P=0.05)	Activity ratio (morphine=1)
Morphine	2.0	1.7–2.4	1
Diamorphine	0.04	0.02–0.06	50
Phenadoxone	0.05	0.03–0.07	40
Pethidine	2.0	1.8–2.2	1
Ketobemidone	0.62	0.47–0.64	3.2
Amidone	0.31	0.20–0.80	6.5

TABLE III

The intravenous potency of analgesic drugs in rats to a contact heat stimulus, based on the " duration " of maximum analgesic effect

Drug	AD50 (" duration ") mg./kg.	Limits (P=0.05)	Activity ratio (morphine=1)
Morphine	2.2	1.9–2.8	1
Diamorphine	0.11	0.06–0.12	20
Phenadoxone	0.10	0.09–0.14	22
Pethidine*	—	— —	—
Ketobemidone	0.69	0.59–0.82	3.2
Amidone	0.48	0.40–1.4	4.6

* In no single animal over the dose range investigated did the analgesic effect persist for 15 minutes.

Fig. 5 and Table III for the same dose ranges as in Fig. 4. Diamorphine and phenadoxone are still much more effective drugs in this sense than the others. The surprising failure of pethidine to produce a sustained response has already been mentioned ; otherwise the analgesics maintain the same order of potency as in Table II.

In view of the enhanced potency of diamorphine and phenadoxone by the intravenous route it seemed of interest to determine the relative toxicities of the analgesics under investigation by the same route. The results obtained (Table IV) illustrate the

TABLE IV

INTRAVENOUS TOXICITY OF ANALGESIC DRUGS

Drug	Dose mg./kg.	24 hr. deaths	% mortality
Morphine	40	0/5	0
	60	0/5	0
	80	0/5	0
	100	1/5	20
	120*	2/5	40
Diamorphine	5	0/5	0
	10	0/5	0
	20	2/5	40
	30	4/5	80
	35	5/5	100
	50	5/5	100
Phenadoxone	6.25	0/5	0
	12.5	2/5	40
	18.75	5/5	100
	25.0	5/5	100
Pethidine	12.5	0/5	0
	17.5	0/5	0
	20.0	1/5	20
	25.0	3/5	60
	40.0	5/5	100
Ketobemidone	2.5	0/5	0
	5.0	2/5	40
	12.5	3/5	60
	15.0	4/5	80
	25.0	5/5	100

* Limit of solubility at the intravenous dose volume of 0.1 ml./100 g. wt.

well-known tolerance of the rat for morphine, as it was impossible for solubility reasons to administer a dose greater than 120 mg./kg. and maintain the same dose volume as had been used throughout. The LD50 appeared to be about 140 mg./kg. The other drugs examined were much more toxic, their dose-response curves were steep and lay quite close together. The LD50 (mg./kg.) values were as follows : diamorphine, 22.5 ; pethidine, 22.5 ; phenadoxone, 12.5 ; and ketobemidone, 10. That diamorphine and phenadoxone do not show any relative enhancement of toxicity by the intravenous route suggests that the analgesic effects of drugs in the rat is not a manifestation of general toxicity. It would appear, moreover, that diamorphine and phenadoxone should be safer drugs to use intravenously for the relief of pain. It would be very interesting to have clinical observations on the relative efficacy of analgesics by intravenous administration.

The main object of this work was to devise some technique which would enable a comparison of the thermal responses of man and the rat to be made, and the comparative efficacy of analgesic drugs to be determined. It appears, however, that the method described in this paper possesses some advantages over techniques described by previous workers. The results obtained with a similar technique applied to man appear in a subsequent paper.

SUMMARY

1. A technique is described for evaluating the potency of analgesic drugs in the rat, using a thermal stimulus and measuring the temperature of reaction.

2. The normal temperature of reaction is about 40° C. Under the influence of an analgesic drug the temperature of reaction may rise up to a maximum of about 48° C. Beyond this temperature tissue damage occurs. Should no tail reaction have occurred when 48° C. is reached, it is unlikely to be elicited at higher temperatures. Attainment of this temperature is thus regarded as a maximum " analgesic " effect.

3. The intravenous potencies of various analgesic drugs are compared in terms of intensity and duration of effect. After intravenous injection of one of these drugs the maximum effect is reached almost invariably within one minute.

4. Whereas administration of morphine, pethidine, and amidone intravenously appears to have little effect on the analgesic potency, compared with the subcutaneous route, diamorphine and phenadoxone show greatly enhanced activity when injected intravenously, both as regards intensity and duration of effect.

5. The intravenous toxicities of morphine, diamorphine, phenadoxone, pethidine, and ketobemidone have been estimated. The enhanced analgesic potency of diamorphine and phenadoxone by the intravenous route is not due to any relative increase in toxicity compared with the other analgesics.

It is a pleasure to acknowledge indebtedness to Professor A. D. Macdonald, at whose suggestion this work was undertaken and for his continued interest in its progress.

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