The effect of narcotics and narcotic antagonists on the tail-flick response in spinal mice

Irwin, Houde & others (1951) reported that the tail-flick response of rats to radiant heat has the characters of a simple reflex. The ability of spinal rats to respond to this stimulus has been confirmed and extended (Winter & Flataker, 1951; Bonnycastle, Cook & Ipsen, 1953). Morphine inhibited the reflex in spinal rats but a quantitative difference observed between its effect in spinal rats and intact animals indicated that a second action might exist in its capacity to increase supraspinal inhibitory mechanisms. We have used the tail-flick test in mice to elucidate the mechanism of action of morphine and the narcotic-antagonist analgesics (Harris & Pierson, 1964; Dewey, Harris & others, 1969; Harris, Dewey & others, 1969) and have found a high correlation of activity with a number of narcotic analgesics to exist between species.

We have confirmed and extended the observation that cholinergic agents such as oxotremorine and physostigmine also reduced this response (Harris & others, 1969; Howes, Harris & others, 1969). In addition, we have shown that an increase in central adrenergic or 5-hydroxytryptamine tone will increase the activity of morphine in intact mice in the tail-flick test (Dewey, Harris & others, 1968). We have now attempted to increase our knowledge about this testing procedure by studying the narcotics, the narcotic antagonists, and some of the neurochemicals discussed above in spinal mice.

Male albino mice of the Swiss-Webster strain (18–25 g) had transections made under ether anaesthesia. A dorsal midline incision was made and the spinal cord was exposed between the fifth and sixth thoracic vertebrae. The cord was cauterized and the wound was closed with silk sutures. Attempts to transect the cord at a higher level resulted in death from respiratory paralysis or uncontrolled bleeding. There were few deaths from the surgical procedure. Water and food were presented *ad libitum*. Within 3 to 4 h all mice were quite active. Simple physiological stimuli showed that the cord section was positive. Most of the mice responded to the radiant stimulus of the tail-flick apparatus within 4 s, the variability among the reaction times being less than is usually observed in normal mice. Animals not responding within 4 s were not used. The results obtained were averaged with a second reading taken 30 min later, after which the drug was given subcutaneously in the flank; readings were made 20 min later. Mice not responding within 10 s were removed from the apparatus and considered to be 100% affected. The % maximal possible inhibition was calculated using the following formula:

$$\frac{\text{test}-\text{control}}{10-\text{control}} \times 100 = \% \text{ maximal possible inhibition}$$

The ED50 for morphine in intact mice is 6·25 mg/kg in this procedure, in our hands. In intact animals, 10 mg/kg of morphine sulphate usually gives near 100% maximum possible inhibition as do all the higher doses used in these experiments.

The results in Table 1 show the effect of morphine on spinal mice. It is much less active than in intact mice. Irwin & others (1951) found a similar decrease in its potency in spinal rats. They concluded that it had an inhibitory effect on the spinal reflex and a stimulatory effect on supraspinal inhibitory processes. These supraspinal processes were removed in the spinal animals explaining the observed decrease in potency of morphine. Our mouse evidence differs from the findings with spinal rats in that increasing the dose of morphine to a high level did not increase its activity in mice. In spinal mice as opposed to spinal rats there appears to be a limit to the ability of morphine to inhibit the reflex.

Table 1. *The effects of morphine and naloxone on the tail-flick test in spinal mice*

Drug	Dose mg/kg	N	Control time (s)	Test time (s)	% maximal possible inhibition
Morphine	10	19	2·6	3·5	18
Morphine	20	23	2·8	4·6	32
Morphine + naloxone	20 1	13	2·2	2·5	3
Morphine	40	30	2·4	3·6	16
Morphine + naloxone	40 1	19	2·1	2·1	0
Morphine	62·5	17	2·3	3·6	18
Morphine + naloxone	62·5 1	10	2·2	2·0	0
Morphine	120	8	2·7	3·2	7

Naloxone, a nearly pure antagonist (Blumberg, Dayton & Wolf, 1966), antagonized the activity of morphine (Table 1). This indicates that, in this procedure, the antagonist's activity is not limited to an effect on supraspinal mechanisms. The narcotic-antagonist analgesics, pentazocine and cyclazocine, at normal doses were almost without activity in the tail-flick test in intact mice. In spinal mice, at very high doses where some activity is seen for cyclazocine in normal animals (Harris & Pierson, 1964), pentazocine (60 mg/kg) and cyclazocine (100 mg/kg) caused a 19 and 27% maximum inhibition in spinal mice. There was complete inhibition in a few of the animals but no effect on most. Morphine on the other hand delayed the reaction time for most mice.

As there are cholinergic inhibitory cells in the spinal cord (Eccles, 1964) it might be expected that the activity of physostigmine and oxotremorine in normal mice would be largely due to spinal inhibition. If this were so these drugs should be relatively equally active in spinal mice. Physostigmine (0·4 mg/kg) and oxotremorine (0·1 mg/kg), both of which cause greater than 80% inhibition in intact mice, gave 13 and 10% maximum possible inhibition in spinal mice, indicating that other central cholinergic mechanisms may play a role in the action of compounds, like the narcotic analgesics, active in this test.

This evidence from the analgesic and the cholinergic drugs indicates that although a spinal reflex is involved in this procedure, the supraspinal influences might be more important than effects at the lower level of the cord. Additional evidence for this point of view is provided by the very high correlation shown to exist between activity in the tail-flick test and addiction potential in man (Archer, Harris & others, 1964).

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The effects of reserpine on the distribution of [^{14}C]5-hydroxytryptamine in the rat

It has been stated that in the rat, as in many other species, reserpine facilitates the oxidative deamination of both endogenously bound and parenterally administered 5-hydroxytryptamine (5-HT) (Erspamer, 1956a; Garattini, Lamesta & others, 1961; Airaksinen, 1963; Axelrod & Inscoe, 1963; Snyder, Wurtman & others, 1964). The results to be presented in this letter are not entirely consistent with these observations.

Male Wistar rats of 230 $\pm$ 10 g were treated intraperitoneally with reserpine (5 mg/kg) or the vehicle solution (20% ascorbic acid). Eighteen h later they were anaesthetized with pentobarbitone, pithed (Shipley & Tilden, 1947) and given a 1 min infusion of 4·08 μg (4 μCi) of [^{14}C]5-HT creatinine sulphate monohydrate into the femoral vein. At the end of the infusion, a plasma sample and heart and kidney tissues were processed for their total radioactivity and unchanged [^{14}C]5-HT content by the methods previously described (Fozard, 1969). The results are shown in Fig. 1.

There was no significant difference as a result of reserpine pretreatment in the total radioactivity levels of plasma, heart or kidney, or in the unmetabolized [^{14}C]5-HT content of heart and kidney. The small sample of plasma obtainable during the collection period did not allow routine determination of the unmetabolized [^{14}C]5-HT content of the plasma total radioactivity. The tissue to plasma ratios of total radioactivity (Weiner & Trendelenburg, 1962) for the ascorbic acid and reserpine pretreated groups respectively were 0·9 $\pm$ 0·10 and 0·92 $\pm$ 0·13 for hearts and 1·74 $\pm$ 0·24 and 2·14 $\pm$ 0·41 for kidneys.

The rapid metabolism of [^{14}C]5-HT accumulated by hearts and kidneys of reserpine pretreated rats has been shown to be the result of oxidative deamination by monoamine oxidase (Fozard, 1969), and was predictable from the earlier observations of Erspamer (1956a), Airaksinen (1963) and Axelrod & Inscoe (1963). However, in the present work an unexpected finding was that the results obtained in animals given reserpine were not significantly different from those obtained in the vehicle-pretreated controls. The explanation may be related to the dose of 5-HT used and its mode of administration.

Both Erspamer (1956b) and Airaksinen (1963) demonstrated that in normal rats the proportion of a small dose of amine excreted as 5-hydroxyindoleacetic acid was greater than that excreted from a larger dose. Airaksinen (1963) found the proportion of deaminated metabolites when 5-HT was given subcutaneously or by slow intravenous injection was greater than when given by rapid intravenous injection. In his experiments, pretreatment with reserpine increased the amount of 5-hydroxyindoleacetic acid after intravenous injection of 5-HT such that the difference usually observed