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Monoamine mediation of the morphine-induced activation of mice

BERNARD J. CARROLL* AND PETER T. SHARP

Department of Psychiatry, University of Melbourne, Victoria 3050, Australia

Summary

1. The dose-response relationship for hyperactivity in grouped mice following the injection of morphine sulphate has been established.

2. The activation response can be modified by drugs which affect either catecholamines or indoleamines.

3. The monoamine precursors L-DOPA and 5-hydroxytryptophan potentiate the response.

4. The monoamine synthesis inhibitors α-methyl-*p*-tyrosine and *p*-chlorophenylalanine reduce the response.

5. Inhibition of monoamine oxidase activity by pargyline caused a great increase in the response. The simultaneous administration of reserpine resulted in a further potentiation.

6. Reserpine blocked the response whenever it was given alone, either before, with or after the injection of morphine.

7. Blockade of α-adrenoceptors with phentolamine or phenoxybenzamine reduced the response.

8. Blockade of tryptaminergic receptors with methysergide or cinanserin also antagonized the response.

9. The major tranquillizers haloperidol and chlorpromazine reduced the response. Haloperidol was especially effective in this regard.

10. The tricyclic antidepressant drug imipramine potentiated the response.

11. The morphine antagonist nalorphine completely prevented the response.

12. The anticholinergic agent atropine and the antihistaminic drug mepyramine did not affect the response.

13. We conclude that dopamine, noradrenaline and 5-hydroxytryptamine are all involved in the normal activation response of grouped mice to morphine, with dopaminergic mechanisms being of primary importance.

Introduction

The mode of action of morphine in producing analgesia has not been established with certainty, although it is clear that the effect is at least partly mediated by cerebral monoamines. Both catecholamines and indoleamines seem to be involved, depending on the experimental conditions chosen (Vedernikov & Afrikanov, 1969 ; Fennessy & Lee, 1970).

*Present address: Depression Research Unit, University of Pennsylvania, Department of Psychiatry, Veterans Administration Hospital, Philadelphia, Pa. 19104, U.S.A.

Another characteristic central action of morphine is the induction of locomotor hyperactivity in some species, notably the cat (Guinard, 1890) and the mouse (Eddy, 1941). A number of reports indicate that this activation response can be modified by drugs which affect cerebral monoamines, but a systematic study of the phenomenon has not been carried out. The most detailed information available is that given by Hollinger (1969) who measured levorphanol-induced running activity in isolated mice. Reserpine and α-methyl-*p*-tyrosine antagonized the response, while pargyline caused a potentiation and *p*-chlorophenylalanine was without effect. Hollinger concluded from these results that the activation response of isolated mice to levorphanol was mediated through the central adrenergic system and that tryptaminergic mechanisms were not important.

We have tested several classes of drugs, with both specific and nonspecific actions on cerebral monoamines, for their ability to modify the activation induced by morphine in grouped mice. Our results indicate that both the catecholamines and the indoleamines are involved in mediating this response and that dopamine is probably the catecholamine of major importance.

Methods

Male albino Swiss mice (Commonwealth Serum Laboratory, Melbourne, strain) weighing between 20 and 24 g were housed in groups of six, in boxes of dimensions $28 \times 16 \times 10$ cm for at least 5 days before testing. The boxes were routinely cleaned 60 min before the injections of morphine. Naïve mice were used in all experiments. Activity was measured by visual counting of the number of incursions made by the mice into a defined area of the living box. Three consecutive one minute activity counts were made at 30 min intervals for 120 min after the injections of morphine. The activity patterns obtained in this way compared closely with those given by continuous recording with an Animex activity meter (AB Farad, Sweden) (Svensson & Thieme, 1969).

In each experiment three groups of mice were observed simultaneously: (1) a group which received morphine after pretreatment with the test drug; (2) a group which received saline after pretreatment with the test drug; (3) a group which received morphine after pretreatment with saline or suspension vehicle. Thus two control groups were included in each experiment. An additional control group (which received two saline injections) was not included after the initial trials since the basal running activity of the mice during the day was very low. At least three experiments were conducted for each test drug.

Drugs

All drugs were injected intraperitoneally. The doses described below are those of the respective salts.

After the dose-response relationship was established 25 mg/kg of morphine sulphate was used in all trials (see below).

The following drugs were prepared in 0·9% NaCl w/v (saline) and were injected 30 min before morphine: atropine sulphate, 2 and 10 mg/kg; chlorpromazine HCl, 1·0 mg/kg (May & Baker Ltd.); haloperidol, 0·5 mg/kg (G. D. Searle Pty. Ltd.); L-5-hydroxytryptophan, 50 mg/kg (Calbiochem Inc.); imipramine hydrochloride, 50 and 100 mg/kg (Geigy Pharmaceuticals Pty. Ltd.); mepyramine

maleate, 25 mg/kg (May & Baker Ltd.); nalorphine hydrochloride, 5 mg/kg (Burroughs Wellcome & Co. Ltd.); phenoxybenzamine hydrochloride, 10 mg/kg (Smith, Kline & French Laboratories Ltd.); phentolamine mesylate, 10 mg/kg (Ciba Ltd.); (±)-propranolol hydrochloride, 10 mg/kg (I.C.I. Ltd.); L-tryptophan, 50 mg/kg (Calbiochem Inc.).

The drugs for which other schedules and preparations were employed are described below.

α-Methyl-*p*-tyrosine (αMPT) (Calbiochem Inc.) was suspended in water with carboxymethylcellulose 0·5% and Tween 80 2·5%. It was given in three doses of 100 mg/kg at 21, 15 and 3 h before morphine (Menon, Dandiya & Bapna, 1967). An identical control vehicle was prepared for use in place of saline.

Cinanserin (2′-(3-dimethylaminopropylthio) cinnamanilide HCl) (Squibb & Sons Pty. Ltd.) was dissolved in saline and given as a dose of 5 mg/kg. In separate experiments the dose was given either 30 min before morphine or together with morphine.

L-Dihydroxyphenylalanine (DOPA) (Hoffmann–La Roche & Co. Ltd.) was suspended in the vehicle described above and was given as two injections of 250 mg/kg, the first 30 min before and the second together with morphine. A fresh suspension of DOPA was required for each experiment since oxidation occurred, with darkening on exposure to light.

Methysergide maleate (Sandoz AG) was given in separate experiments at two dosage levels, 2·5 and 5 mg/kg. Each dose was given once daily for two days before morphine, as well as 30 min before morphine on the experimental day.

p-Chlorophenylalanine (*p*CPA) (Calbiochem Inc.) was suspended in the vehicle described above and was given as a single dose of 100 mg/kg three days before morphine administration (Miller, Cox, Snodgrass & Maickel, 1970).

Pargyline HCl (Abbott Laboratories) was dissolved in saline and given three hours before morphine in a dose of 75 mg/kg.

Reserpine (Ciba Company Pty. Ltd.) was in ampoules supplied by the manufacturer. The vehicle was water with polyethylene glycol, citric acid and benzyl alcohol. A comparable control vehicle was not used in the experiments with reserpine.

Statistical comparisons

As the control groups usually showed maximum activity 60 min after receiving morphine, all other activity counts were expressed as percentages of this level, after the raw activity scores from all experiments with each test drug had been combined. For n experiments with a test drug there were $3 \times n$ one minute activity counts at 60 min for both the drug-treated and control groups. The percentage means (±S.D.) at 60 min were compared by Student's t test. The figures reveal that there was no advantage in using the activity levels at other times after morphine in addition to the sixty minute results.

Results

Behavioural response

After the injection of morphine to mice a decrease in spontaneous movement is observed, which lasts for 10 to 15 minutes. Activation then occurs and the

animals begin to run from one end of the box to the other and around the perimeter of the box. This response reaches a peak at about 60 min and lasts for at least 2 hours. The rate of running is regular and the behaviour has an automatic, driven, stereotyped quality; it does not resemble exploratory or escape behaviour. The mice do not display aggression and they are easily handled. With very large doses of morphine the response diminishes as the mice become ataxic and convulsions occur (Eddy, 1941). The activation induced by morphine is easily distinguished from the excitement caused by amphetamine, where the running tends to be episodic at high dosage, with periods of tremulous 'freezing', and increased aggression is prominent. Other authors have described the activation of mice caused by opiate alkaloids as a 'running fit' (Goldstein & Sheehan, 1969). From our observations this is an unfortunate term to apply to such a predictable, stereotyped, dose-dependent response.

Associated phenomena which appear after morphine is given are hyperextended tails (Straub, 1911) which develop within a few minutes, and stereotyped digging, sniffing and gnawing which commence during the second hour as the rate of running is decreasing.

TABLE 1. *Raw activity counts of grouped mice following injections of morphine sulphate, 5–225 mg/kg. Activity at each time is expressed as the mean of 6×1 minute counts from two experiments (3 counts in each experiment)*

Dose of morphine sulphate (mg/kg)	Raw activity counts (means) at −30	0	30	60	90	120 min
5	3·5	2·5	2·3	1·8	3·2	1·0
10	2·3	1·8	5·0	5·7	4·0	1·3
25	2·0	2·5	12·0	21·0	11·0	5·2
75	2·8	2·0	45·0	48·0	48·0	33·0
150	3·5	6·5	55·0	66·0	59·0	48·0
225	1·5	18·0	62·0	59·0	48·0	40·0

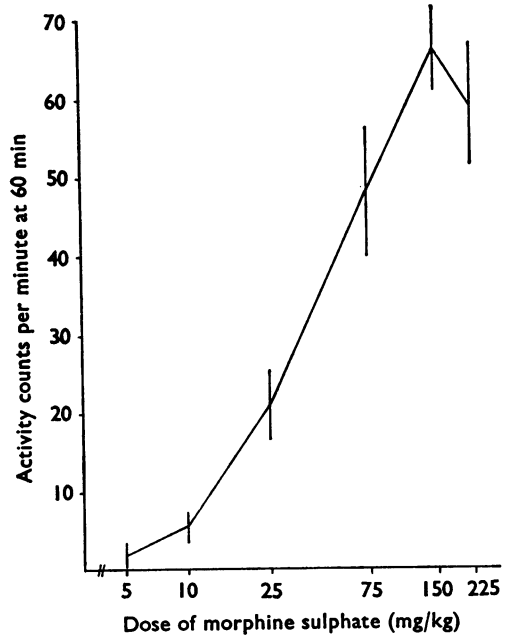

FIG. 1. Dose-response relationship for activation of grouped mice after injection of morphine sulphate, 5–225 mg/kg. Activity of each group at 60 min is given (means of 6×1 minute activity counts from 2 experiments). Vertical bars indicate standard deviations of the means. Morphine sulphate dosage is plotted logarithmically.

Dose-response relationship

The optimal dose of morphine for use in detecting antagonism and potentiation was obtained from a study of the dose-response relationship. For this purpose two experiments were conducted in which six groups of mice received 5, 10, 25, 75, 150 or 225 mg/kg of morphine sulphate. With each dose the maximum activity occurred at 60 minutes (Table 1). The results are shown in Fig. 1 which reveal an approximately sigmoid relationship between the logarithm of the dose and the observed maximum activity.

The dose of 25 mg/kg was chosen as the most appropriate one to use in subsequent experiments. With this dose the maximum activity (21 counts/min) could be accurately measured by visual observation.

The effects of the test drugs against the morphine-induced activation are summarized in Table 2.

Monoamine synthesis inhibitors

Both αMPT and *p*CPA reduced the response to morphine. In the doses used the effect of αMPT (an inhibitor of dopamine (DA) and noradrenaline (NA) synthesis) was greater than that of *p*CPA (an inhibitor of 5-hydroxtryptamine (5-HT) synthesis) (Fig. 2). The brain amine concentrations and the degree of inhibition of tyrosine and tryptophan hydroxylases were not measured.

TABLE 2. *Effects of drugs on morphine-induced activity of grouped mice. The timing of drug administration is given in the text*

Drug	Dose (mg/kg)	% Activity at one hour[1] Mean	S.D.	N[2]	*P*
αMPT	see text	14	13	9	0·001
*p*CPA	100	57	14	9	0·001
DOPA[3]	see text	190	78	9	0·01
5-HTP	50	180	19	12	0·001
Tryptophan	50	109	41	9	—
,,	100	95	24	9	—
Pargyline	75	240	76	9	0·001
Pargyline+ Reserpine	5	420	91	9	0·001
Phentolamine	10	45	32	9	0·001
Phenoxybenzamine	10	43	12	9	0·001
Propranolol	10	110	30	9	—
Cinanserin (0 min)	5	14	3·4	9	0·001
,, (30 min)	5	47	22	9	0·01
Methysergide	2·5	70	7·6	9	0·1
,,	5	39	12	9	0·01
Haloperidol	0·5	1	1	9	0·001
Chlorpromazine	1·0	21	16	9	0·001
Imipramine	50	200	92	9	0·01
,,	100	230	99	12	0·001
Atropine	2	103	17	9	—
,,	10	96	24	9	—
Mepyramine	25	103	63	9	—
Nalorphine	5	1	1	9	0·001

[1] Activity of test groups as percentage of activity of control groups at one hour.
[2] Number of one minute activity counts (=3 × number of experiments with each test drug).
[3] Activities of DOPA-treated and control groups compared at 30 min (see Fig. 3)

Monoamine precursors

Both DOPA and 5-HTP increased the response to morphine to a similar extent (Table 2 and Fig. 3). On the other hand L-tryptophan (50 and 100 mg/kg) did not affect the response to morphine (Table 2).

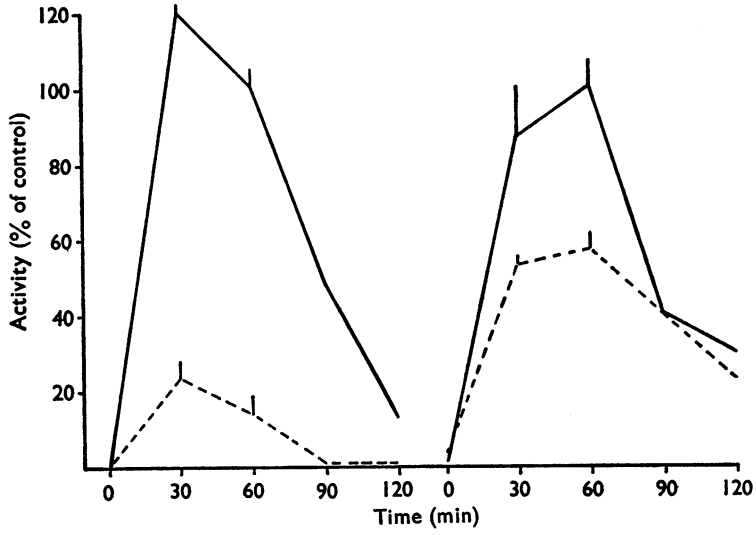

FIG. 2. Effect of pretreatment with αMPT (left) and with *p*CPA (right) on the activation response of grouped mice to morphine sulphate, 25 mg/kg. Activities are expressed as a percentage of the activity of the solvent-pretreated control group at 60 minutes. Vertical bars indicate standard errors of the means. Dosage schedules are given in the text. Solid lines indicate control groups; broken lines indicate drug pretreated groups.

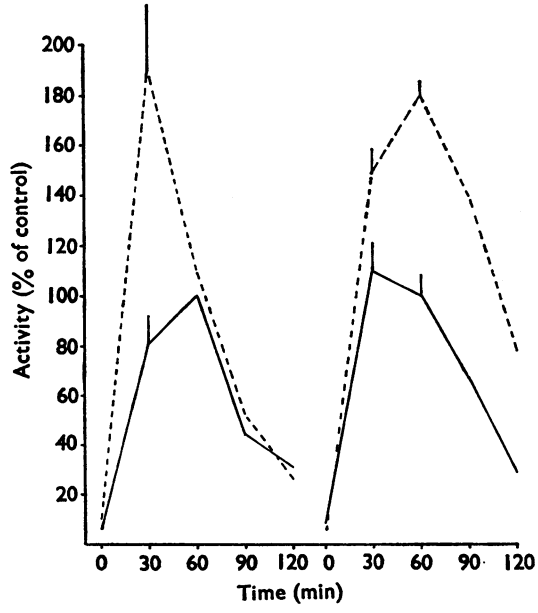

FIG. 3. Effects of pretreatment with L-DOPA (left) and with L-5-HTP (right) on activation response of grouped mice to morphine sulphate, 25 mg/kg. Dosage schedules are given in the text. Vertical bars indicate standard errors of the means. Activities are expressed as in Figure 2.

Inhibition of monoamine oxidase

Previous treatment with pargyline caused a large increase in the activity response to morphine. Simultaneous addition of reserpine at the time of morphine injection increased the response even further (Table 2 & Fig. 4). The combination of pargyline and reserpine, *without* morphine, induced a qualitatively different behaviour, with running, stereotyped rotational movements, aggression and profuse sweating.

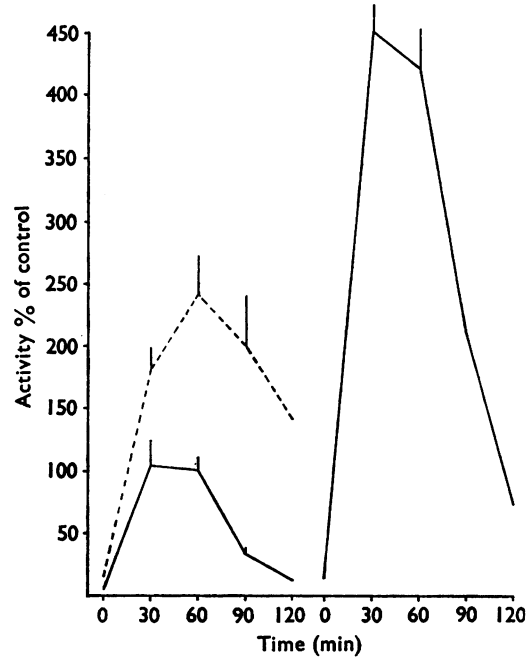

FIG. 4. Effects of pretreatment with pargyline (left) and with pargyline plus reserpine (right) on the activation response of grouped mice to morphine sulphate, 25 mg/kg. Dosage schedules are given in text. Vertical bars indicate standard errors of the means. Activities are expressed as in Figure 2.

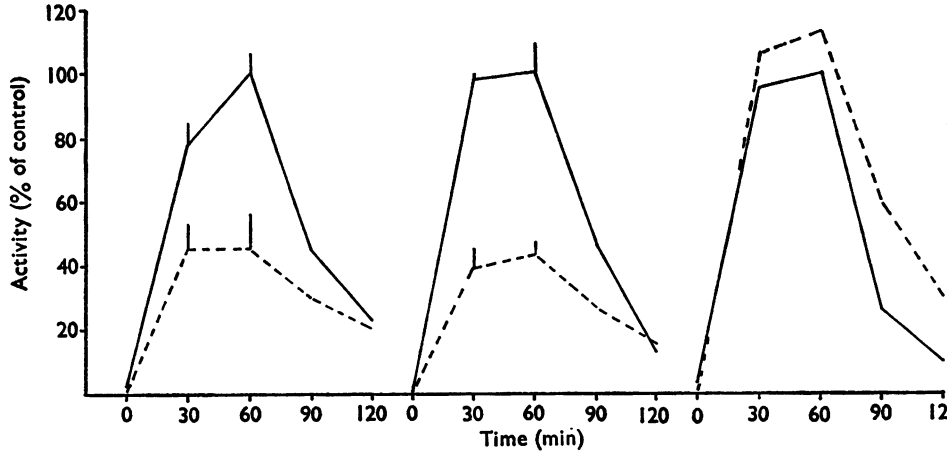

FIG. 5. Effects of pretreatment with phentolamine (left), with phenoxybenzamine (centre) and with propranolol (right) on the activation response of grouped mice to morphine sulphate, 25 mg/kg. Dosage schedules are given in the text. Vertical bars indicate standard errors of the means. Activities are expressed as in Figure 2.

Reserpine

When reserpine (5 mg/kg) was given together with morphine the activation response was prevented and the mice were sedated. When reserpine was given 30 or 60 min after morphine the activity which had started subsided rapidly and no phase of potentiation was seen.

Receptor blockade

The α-adrenoceptor blocking agents phentolamine and phenoxybenzamine each impaired the response to morphine. The β-adrenoceptor blocker propranolol did not affect the response: a small trend towards potentiation was observed (Table 2 and Fig. 5).

Cinanserin and methysergide are drugs which block central and peripheral tryptaminergic receptors (Clineschmidt & Anderson, 1970); both compounds antagonized the response to morphine (Table 2; Fig. 6). The effect of methysergide was dose-dependent; cinanserin was more effective when given together with morphine than it was when given 30 min before morphine.

Major tranquillizers

Haloperidol was the most effective antagonist which we examined. In a dose of 0·5 mg/kg it completely suppressed the activation response. Chlorpromazine (0·1 mg/kg) was less effective, but still produced significant antagonism (Table 2 and Fig. 7).

Imipramine

Imipramine significantly increased the activation response to morphine (Table 2 and Fig. 8). The effects of other tricyclic anti-depressants have also been studied

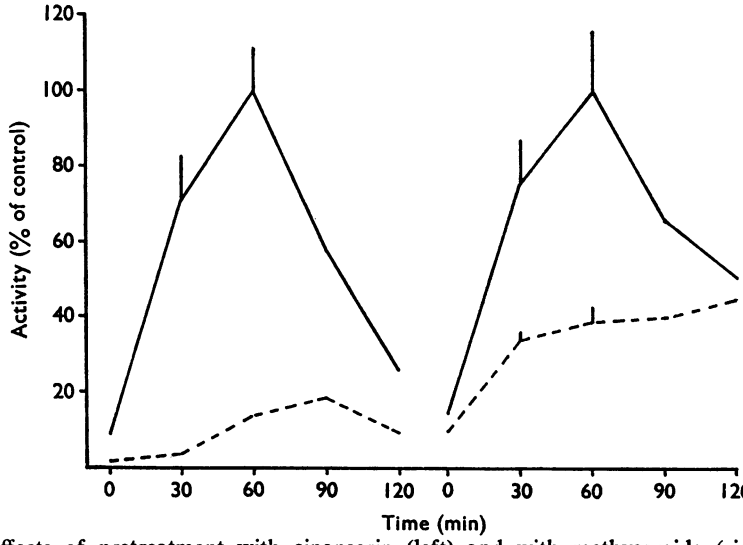

FIG. 6. Effects of pretreatment with cinanserin (left) and with methysergide (right) on the activation response of grouped mice to morphine sulphate, 25 mg/kg. Cinanserin (5 mg/kg) was given together with morphine. Methysergide (5 mg/kg) was given at the times detailed in the text. Vertical bars indicate standard errors of the means. Activities are expressed as in Figure 2.

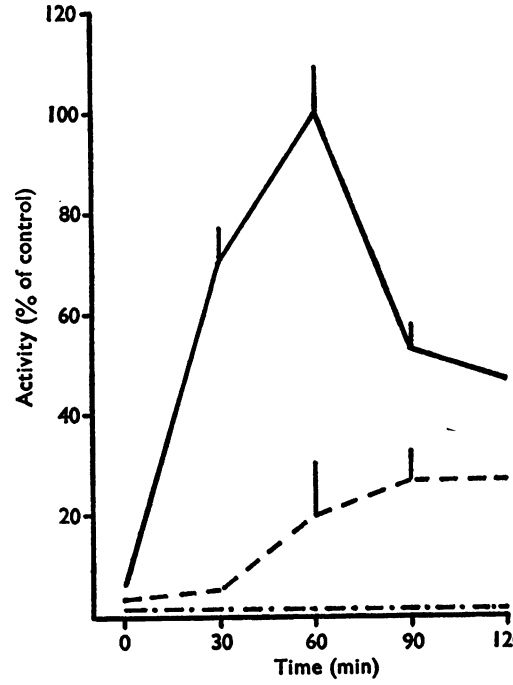

FIG. 7. Effects of pretreatment with chlorpromazine (– – –) and with haloperidol (–·–·–) on the activation response of grouped mice to morphine sulphate, 25 mg/kg. Dosage schedules are given in the text. Vertical bars indicate standard errors of the means. Activities are expressed as in Figure 2.

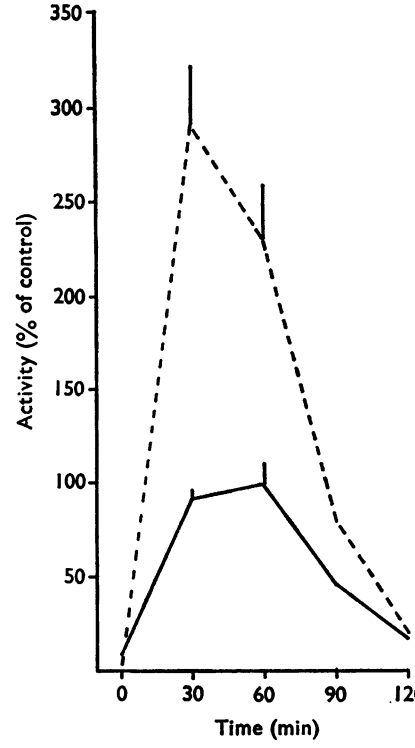

FIG. 8. Effect of pretreatment with imipramine (100 mg/kg) on the activation response of grouped mice to morphine sulphate, 25 mg/kg. The dosage schedule is given in the text. Vertical bars indicate standard errors of the means. Activities are expressed as in Figure 2.

but will not be described in detail. Briefly, desipramine and nortriptyline had an effect similar to that of imipramine, while amitriptyline and doxepin impaired the response to morphine (Sharp & Carroll, in preparation).

Other drugs

The anticholinergic drug atropine did not affect the response to morphine nor did the antihistaminic agent mepyramine (Table 2).

The morphine antagonist nalorphine completely suppressed the activation response when given as a dose of 5 mg/kg 30 min before morphine. At this dose no activation was produced by nalorphine itself.

None of the drugs which antagonized the activation response abolished the Straub tail phenomenon, but no attempt was made to measure this aspect quantitatively.

With the exception of reserpine, none of the drugs which antagonized the activation response caused sedation when given to control groups in the doses described. Sedation caused by the test drugs (group 2 animals) was assessed by direct observation and by the animals' responses to handling and injection. Activity counts were not sufficient for this purpose, since animals which received only two injections of saline were very inactive during these experiments, with count rates between 0 and 2 per minute (i.e. about 5% of the usual maximum activity after morphine).

Discussion

Methodology

It is our experience that, when suitable doses are used, the activation response of mice to morphine is a sensitive index of the drug's action. The activation response, rather than the analgesic effect, has also been used to study the development of tolerance to opium alkaloids in mice (Shuster, Hannam & Boyle, 1963 ; Goldstein & Sheehan, 1969). The dose-activation response relationship which we have established (Fig. 1 and Table 1) is in agreement with the general description given by Eddy (1941) and is close to the dose-response relationship for morphine's effect on catecholamine synthesis in mouse brain (Smith, Villareal, Bednarczyk & Sheldon, 1970). In a study of the effects of cortisone, Winter & Flataker (1951) empirically used the same dose of morphine sulphate as we did (25 mg/kg) and commented that this dose was 'rather high'. In our experiments this dose caused only a moderate activation response (Fig. 1) and was the optimal amount to use for detecting either antagonism or potentiation.

Inhibition of amine synthesis

The finding that inhibition of DA and NA synthesis by αMPT (Spector, Sjoerdsma & Udenfriend, 1965) antagonized the activation response of mice agrees with the reports of Menon, Dandiya & Bapna (1967) and of Hollinger (1969). Rats which are tolerant to morphine also develop activation with each injection and Eidelberg & Schwartz (1970) found that αMPT was an effective antagonist in such animals as well. αMPT has further been found to reduce the spontaneous motor activity of mice (Svensson & Waldeck, 1970) and to antagonize the excitement produced in mice by (+)-amphetamine, cocaine and mescaline (Menon, Dandiya & Bapna, 1967 ; Svensson, 1970).

When indoleamine synthesis was inhibited by *p*CPA we observed a significant reduction in the activation response of mice to morphine (Fig. 2 and Table 2). This finding differs from that of Hollinger (1969), in spite of the fact that we used a much lower dosage of the inhibitor. It was shown by Miller, Cox, Snodgrass & Maickel (1970) that *p*CPA affects the brain levels of NA as well as lowering 5-HT levels. The schedule which we used, taken from their data with rats, was chosen to have minimal effects on brain NA levels, while the 5-HT levels were still depressed. The only other variables which seem to be significant between our experiments and Hollinger's are (a) the strain of mice, (b) the use of morphine instead of levorphanol, and (c) the use of grouped rather than isolated mice. Other workers have obtained evidence that grouping can modify the responses of mice to various drug treatments. For example grouped mice treated with reserpine after monoamine oxidase (MAO) inhibition are more active than grouped controls, while treated isolated mice have the same activity as isolated controls; this effect has been correlated with an increased brain 5-HT level in the grouped mice (Pfeifer & Galambos, 1967). Such an effect of grouping on activity and brain 5-HT levels may help to explain this difference between our results and Hollinger's.

Eidelberg & Schwartz (1970) observed that *p*CPA reversed the sedative effect of morphine in naïve rats. Antagonism of the analgesic effect of morphine following *p*CPA has been reported in mice (Fennessy & Lee, 1970) and in rats (Tenen, 1968) but there is disagreement as to whether *α*MPT has a similar action (Verri, Graeff & Corrado, 1967; Fennessy & Lee, 1970).

Treatment with monoamine precursors

The use of L-DOPA caused a large increase in the response of mice to morphine (Table 2 and Fig. 3) but the duration of the running was not affected. A similar potentiation by DOPA was found by Eidelberg & Schwartz (1970) in tolerant rats which were activated by morphine. When given alone DOPA has a biphasic action on the spontaneous motor activity of mice (Svensson & Stromberg, 1970; Stromberg, 1970) and the dose we used did not cause activation by itself.

The interpretation of this potentiating effect of DOPA presents some difficulty, since it is now clear that DOPA affects tryptaminergic mechanisms as well as raising brain DA levels, and its effects on brain NA levels and turnover are variable (see Carroll, 1971, for detailed references). Most probably the potentiation caused by DOPA results from the increase of brain DA: the activating effect of amphetamine (Svensson, 1970) and of nialamide on mice (Svensson & Waldeck, 1970) depends primarily on brain DA, while brain NA may have a minor role in determining spontaneous motor activity.

The potentiation caused by 5-HTP was almost as great as that seen with DOPA (Table 2 & Fig. 3) while L-tryptophan had no significant effect on the activation response to morphine. We have shown that tryptophan in the doses used increases the levels of 5-HT and of 5-hydroxyindoleacetic acid in rat brain (Carroll & Dodge, 1971) and the lack of effect of tryptophan is hard to understand. In naïve rats 5-HTP is known to potentiate morphine analgesia (Contreras, Tamayo & Weitzman, 1970) but tryptophan apparently does not (Vedernikov & Afrikanov, 1969). Tryptophan loads decrease brain DA and NA levels, probably by competition with tyrosine for transport mechanisms (Sourkes, Murphy & Chavez, 1961; Green, Greenberg & Erickson, 1962), an effect which may well interfere with the

locomotor response. On the other hand, after 5-HTP administration 5-HT accumulates in catecholaminergic neurones and displaces the endogenous DA and NA (Carlsson, 1964 ; Lichtensteiger, Mutzner & Langemann, 1967). These effects of amine precursors on non-homologous amines make the use of precursor-loading a less reliable method than it was previously believed to be for the clarification of drug actions (Carroll, 1971).

Inhibition of monoamine oxidase

Treatment with pargyline more than doubled the activation response to morphine. The basal activity of the pargyline-treated mice was not increased over that of the saline pretreated control groups. The potentiating effect of pargyline is in agreement with the finding of Hollinger (1969). The sedation caused by morphine in naïve rats is also reversed by MAO inhibition with iproniazid (Eidelberg & Schwartz, 1970), but there are conflicting reports of the effect of MAO inhibition on morphine analgesia (Medakovic & Banic, 1964 ; Vedernikov & Afrikanov, 1969).

Reserpine

The acute administration of reserpine (5 mg/kg) together with morphine in the pargyline-treated mice caused the most intense activation which we have observed (Fig. 4 and Table 2), but no potentiation by reserpine was seen when MAO activity was normal. These results are further evidence of a role for cerebral monoamines in the activation response. Conflicting reports of the action of reserpine on morphine analgesia are summarized by Fennessy & Lee (1970).

Receptor blockade

A definite reduction in the activation response of mice was caused by each of the α-adrenoceptor blocking drugs, phentolamine and phenoxybenzamine (Table 2 and Fig. 5). The analgesic effect of morphine in rats may be antagonized by phenoxybenzamine (Heller, Saavedra & Fischer, 1968) and by tolazoline (Contreras & Tamayo, 1966).

The β-adrenoceptor blocking drug, propranolol, had no significant effect on the activation of mice by morphine, although there is no doubt that propranolol in the dose used (10 mg/kg) does enter the central nervous system (Laverty & Taylor, 1968). In a dose of 5 mg/kg propranolol was found to antagonize methamphetamine-induced excitement in mice, as did another β-adrenoceptor blocking drug, INPEA (Estler & Ammon, 1971).

We found that the central tryptaminergic receptor blocking drugs methysergide and cinanserin also antagonized the activation response to morphine (Table 2, Fig. 6). Our finding that methysergide (2·5 and 5 mg/kg) was an effective antagonist may be partly due to the prolonged pretreatment (3 days). Fennessy & Lee (1970) found that a single dose of 5 mg/kg did not affect morphine analgesia in the same strain of mice as we used. On the other hand Galambos, Pfeifer, Gyorgy & Molnar (1967) found that a single dose of 2 mg/kg methysergide increased the activation of mice caused by amphetamine. Methysergide has a low

brain/blood distribution ratio (Doepfner, 1962) and when central rather than peripheral clinical effects are being sought, the dose, frequency and route of methysergide administration are important variables (Haskovec, 1969).

Major tranquillizers

Haloperidol 0·5 mg/kg completely prevented the activation response to morphine while chlorpromazine 1·0 mg/kg also had a significant effect (Table 2, Fig. 7). The comparative potencies of these two drugs suggest that blockade of the central DA receptors by haloperidol caused the activation response to be totally suppressed: haloperidol is ten times more active than chlorpromazine against central dopaminergic mechanisms, while the two drugs have equal central α-adrenergic blocking activity (Andén, Butcher, Corrodi, Fuxe & Ungerstedt, 1970). A similar potency ratio for haloperidol and chlorpromazine has been reported against amphetamine-induced stereotyped behaviour in the rat (Lemberger, Witt, Davis & Kopin, 1970) and against DOPA-induced aggression and excitement in mice (Yen, Katz & Krop, 1970).

Imipramine

The tricyclic antidepressant drug imipramine doubled the activation response of mice to morphine (Table 2 and Fig. 8). This effect was also seen with some other tricyclic drugs (desipramine, nortriptyline) but not with those which have marked sedative properties (doxepin, amitriptyline). We have found no other reports dealing with the effect of tricyclic antidepressants on morphine activation or analgesia.

Imipramine is known to block the neuronal re-uptake of NA but does not greatly affect the re-uptake of DA (Carlsson, Fuxe, Hamberger & Lindquist, 1966; Snyder, Green & Hendley, 1968). It also blocks the uptake of 5-HT (Wise & Ruelius, 1968; Carlsson, 1970) and these actions may partly explain why it potentiates the activation response to morphine. An alternative explanation may be that it prolongs the metabolism of morphine: both desipramine and iprindole potentiate the response of rats to amphetamine; the latter drug does not affect amine re-uptake mechanisms but both are known to lessen the rate of metabolism of amphetamine (Miller, Freeman, Dingell & Sulser, 1970; Lemberger, Sernatinger & Kuntzman, 1970).

Other drugs

The anticholinergic drug atropine when given in a dose of either 2 or 10 mg/kg had no effect on the activation response to morphine (Table 2). Atropine is known to have no effect on the spontaneous motor activity of mice but to reduce their exploratory behaviour (Ahtee & Shillito, 1970).

The antihistaminic agent mepyramine was tested because histaminergic mechanisms are involved in the 'sham rage' produced by morphine in dogs (Fennessy & Ortiz, 1968) and in the motor activation which intraventricular infusions of morphine cause in the rabbit (Tanaka & Lin, 1969). As mepyramine was found to have no effect on the activation response of mice (Table 2), a role for histaminergic mechanisms in this response is unlikely.

Nalorphine, which prevents the analgesic effect of morphine also completely

blocked the activation response (Table 2), a finding which suggests that the neuronal receptors for the two actions may be similar (Cox & Weinstock, 1964).

From the sum of these results it seems that noradrenergic and tryptaminergic mechanisms have a role in determining the intensity of the activation response of mice to morphine. The striking suppression which haloperidol produced suggests that dopaminergic mechanisms are essential for the initiation of the response.

These conclusions from the study of drug interactions agree with what is known of the *acute* neurochemical effects of morphine administration. In the naïve mouse morphine increases the turnover of total catecholamines, but not after tolerance to the activating effect has developed (Smith, Villareal, Bednarczyk & Sheldon, 1970). The turnover of DA but not of NA is also increased in rat brain by acute morphine administration. Gunne, Jonsson & Fuxe (1969) observed that this effect was diminished after the development of tolerance whereas Clouet & Ratner (1970) found that the effect was even more marked when tolerance had developed, at which time the behavioural activation also occurs in rats (Eidelberg & Schwartz, 1970). The acute administration of morphine is said not to alter the rate of turnover of 5-HT in mouse brain (Way, Loh & Shen, 1968) although the brain 5-HT concentrations may be reduced, together with those of NA (Lee & Fennessy, 1970).

The major neurochemical response to the acute administration of morphine therefore appears to be an increased DA turnover, with secondary involvement of noradrenergic and tryptaminergic mechanisms. These findings support our conclusion that dopaminergic mechanisms are of primary importance for initiating the activation response of mice to morphine.

REFERENCES

AHTEE, L. & SHILLITO, E. (1970). The effect of benzodiazepines and atropine on exploratory behaviour and motor activity of mice. *Br. J. Pharmac.*, **40**, 361–371.

ANDÉN, N. E., BUTCHER, S. G., CORRODI, H., FUXE, K. & UNGERSTEDT, U. (1970). Receptor activity and turnover of dopamine and noradrenaline after neuroleptics. *Eur. J. Pharmac.*, **11**, 303–314.

CARLSSON, A. (1964). Functional significance of drug-induced changes in brain monoamine levels. In: *Progress in Brain Research, Vol.* 8, *Biogenic Amines*, ed. Himwich, H. E., & Himwich, W. A., pp. 9–27. Amsterdam: Elsevier.

CARLSSON, A. (1970). Structural specificity for inhibition of (^{14}C)-5-hydroxytryptamine uptake by cerebral slices. *J. Pharm. Pharmac.*, **22**, 729–732.

CARLSSON, A., FUXE, K., HAMBERGER, B. & LINDQVIST, M. (1966). Biochemical and histochemical studies on the effects of imipramine-like drugs and (+) amphetamine on central and peripheral catecholamine neurons. *Acta physiol. scand.*, **67**, 481–497.

CARROLL, B. J. (1971). Monoamine precursors in the treatment of depression. *Clin. Pharmac. Ther.*, **12**, 743–761.

CARROLL, B. J. & DODGE, J. (1971). L-Tryptophan as an antidepressant. *Lancet*, **1**, 915.

CLINESCHMIDT, B. V. & ANDERSON, E. G. (1970). The blockade of bulbospinal inhibition by 5-hydroxytryptamine antagonists. *Exper. Brain Res.*, **11**, 175–186.

CLOUET, D. H. & RATNER, M. (1970). Catecholamine biosynthesis in brains of rats treated with morphine. *Science*, **168**, 854–856.

CONTRERAS, E. & TAMAYO, L. (1966). Effects of drugs acting in relation to sympathetic functions on the analgesic action of morphine. *Arch. Int. Pharmacodyn.*, **160**, 312–320.

CONTRERAS, E., TAMAYO, L. & WEITZMAN, P. (1970). Reduction of the antinociceptive effect of 5-hydroxytryptophan in morphine-tolerant rats. *Psychopharmacologia*, **17**, 314–319.

COX, B. M. & WEINSTOCK, M. (1964). Quantitative studies of the antagonism by nalorphine of some of the actions of morphine-like analgesic drugs. *Br. J. Pharmac.*, **22**, 289–300.

DOEPFNER, W. (1962). Biochemical observations on LSD-25 and deseril. *Experientia*, **18**, 256–257.

EDDY, N. B. (1941). Morphine. Action on the nervous system. The Pharmacology of the Opium Alkaloids, ed. Krueger, H., Eddy, N. B., & Sumwalt, M. pp. 1–76. U.S. Public Health Service, Suppl. No. 165. Washington.

EIDELBERG, E. & SCHWARTZ, A. S. (1970). Possible mechanism of action of morphine on brain. *Nature*, **225**, 1152–1153.

ESTLER, C. J. & AMMON, H. P. T. (1971). Modification by two beta-adrenergic blocking drugs of the effects of methamphetamine on behaviour and brain metabolism of mice. *J. Neurochem.*, **18**, 777–779.

FENNESSY, M. R. & LEE, J. R. (1970). Modification of morphine analgesia by drugs affecting adrenergic and tryptaminergic mechanisms. *J. Pharm. Pharmac.*, **22**, 930–935.

FENNESSY, M. R. & ORTIZ, A. (1968). The behavioural and cardiovascular actions of intravenously administered morphine in the conscious dog. *Eur. J. Pharmac.*, **3**, 177–185.

GALAMBOS, E., PFEIFER, A. K., GYORGY, L. & MOLNAR, J. (1967). Study of the excitation induced by amphetamine, cocaine and alpha-methyltryptamine. *Psychopharmacologia*, **11**, 122–129.

GOLDSTEIN, A. & SHEEHAN, P. (1969). Tolerance to opioid narcotics. I. Tolerance to the 'running fit' caused by levorphanol in the mouse. *J. Pharmac. exp. Ther.*, **169**, 175–184.

GREEN, H., GREENBERG, S. M. & ERICKSON, R. W. (1962). Effect of dietary phenylalanine and tryptophan upon the rat brain amine levels. *J. Pharmac. exp. Ther.*, **136**, 174–178.

GUINARD, L. (1890). Action physiologique de la morphine chez le chat. *C.R. Acad. Sci. (Paris)*, **111**, 981–983.

GUNNE, L. M., JONSSON, J. & FUXE, K. (1969). Effects of morphine intoxication on brain catecholamine neurons. *Eur. J. Pharmac.*, **5**, 338–342.

HASKOVEC, L. (1969). Methysergide in mania. *Lancet*, **2**, 902.

HELLER, B., SAAVEDRA, J. M. & FISCHER, E. (1968). Influence of adrenergic blocking agents upon morphine and catecholamine analgesic effect. *Experientia*, **24**, 804–805.

HOLLINGER, M. (1969). Effect of reserpine, α-methyl-*p*-tyrosine, *p*-chlorophenylalanine and pargyline on levorphanol-induced running activity in mice. *Arch. int. Pharmacodyn.*, **179**, 419–424.

LAVERTY, R. & TAYLOR, K. M. (1968). Propranolol uptake into the central nervous system and the effect on rat behaviour and amine metabolism. *J. Pharm. Pharmac.*, **20**, 605–609.

LEE, J. R. & FENNESSY, M. R. (1970). The relationship between morphine analgesia and the levels of biogenic amines in the mouse brain. *Eur. J. Pharmac.*, **12**, 65–70.

LEMBERGER, L., SERNATINGER, E. & KUNTZMAN, R. (1970). Effect of desmethylimipramine, iprindole and dl-erythro-a-(3,4-dichlorophenyl)-B-(*t* butyl amino) propanol HCl on the metabolism of amphetamine. *Biochem. Pharmac.*, **19**, 3021–3028.

LEMBERGER, L., WITT, E. D., DAVIS, J. M. & KOPIN, I. J. (1970). The effects of haloperidol and chlorpromazine on amphetamine metabolism and amphetamine stereotype behaviour in the rat. *J. Pharmac. exp. Ther.*, **174**, 428–433.

LICHTENSTEIGER, W., MUTZNER, U. & LANGEMANN, H. (1967). Uptake of 5-hydroxytryptamine and 5-hydroxytryptophan by neurons of the central nervous system normally containing catecholamines. *J. Neurochem.*, **14**, 489–497.

MEDAKOVIC, M. & BANIC, B. (1964). The action of reserpine and α-methyl-*m*-tyrosine on the analgesic effect of morphine in rats and mice. *J. Pharm. Pharmac.*, **16**, 198–206.

MENON, M. K., DANDIYA, P. C. & BAPNA, J. S. (1967). Modification of the effect of some central stimulants in mice pretreated with α-methyl-l-tyrosine. *Psychopharmacologia*, **10**, 437–444.

MILLER, F. P., COX, R. H., SNODGRASS, W. R. & MAICKEL, R. P. (1970). Comparative effects of *p*-chlorophenylalanine, *p*-chloroamphetamine and *p*-chloro-N-methylamphetamine on rat brain norepinephrine, serotonin and 5-hydroxyindoleacetic acid. *Biochem. Pharmac.*, **19**, 435–442.

MILLER, K. W., FREEMAN, J. J., DINGELL, J. V. & SULSER, F. (1970). On the mechanism of amphetamine potentiation by iprindole. *Experientia*, **26**, 863–864.

PFEIFER, A. K. & GALAMBOS, E. (1967). Toxicity and the action of reserpine on brain monoamines after pretreatment with monoamine oxidase inhibitors in isolated and aggregated mice. *Psychopharmacologia*, **11**, 130–135.

SHUSTER, H., HANNAM, R. & BOYLE, W. (1963). A simple method for producing tolerance to dihydromorphinone in mice. *J. Pharmac. exp. Ther.*, **140**, 149–154.

SMITH, C. B., VILLAREAL, J. E., BEDNARCZYK, J. H. & SHELDON, M. I. (1970). Tolerance to morphine-induced increases in (^{14}C) catecholamine synthesis in mouse brain. *Science*, **170**, 1106–1108.

SNYDER, S. H., GREEN, A. I. & HENDLEY, E. D. (1968). Kinetics of H^3-norepinephrine accumulation into slices from different regions of the rat brain. *J. Pharmac. exp. Ther.*, **164**, 90–102.

SOURKES, T. L., MURPHY, G. F. & CHAVEZ, B. (1961). The action of some α-methyl and other amino acids on cerebral catecholamines. *J. Neurochem.*, **8**, 109–115.

SPECTOR, S., SJOERDSMA, A. & UDENFRIEND, S. (1965). Blockade of endogenous norepinephrine synthesis by α-methyl-tyrosine, an inhibitor of tyrosine hydroxylase. *J. Pharmac. exp. Ther.*, **147**, 86–95.

STRAUB, W. (1911). Eine empfindliche biologische Reaktion auf Morphin. *Deutsche med. Wochenschrift*, **37**, 1462.

STROMBERG, U. (1970). DOPA effects on motility in mice; potentiation by MK 485 and dexchlorpheniramine. *Psychopharmacologia*, **18**, 58–67.

SVENSSON, T. H. (1970). The effect of inhibition of catecholamine synthesis on dexamphetamine induced central stimulation. *Eur. J. Pharmac.*, **12**, 161–166.

SVENSSON, T. H. & STROMBERG, U. (1970). Potentiation by amantadine hydrochloride of l-DOPA induced effects in mice. *J. Pharm. Pharmac.*, **22**, 639–640.

SVENSSON, T. H. & THIEME, G. (1969). An investigation of a new instrument to measure motor activity of small animals. *Psychopharmacologia*, **14**, 157–163.

SVENSSON, T. H. & WALDECK, B. (1970). On the role of brain catecholamines in motor activity: experiments with inhibitors of synthesis and of monoamine oxidase. *Psychopharmacologia*, **18**, 357–365.

TANAKA, K. & LIN, Y. (1969). Histamine liberation into the cerebrospinal fluid by drugs applied intraventricularly. *Jap. J. Pharmac.*, **19**, 510–514.

TENEN, S. S. (1968). Antagonism of the analgesic effect of morphine and other drugs by *p*-chlorphenylalanine, a serotonin depletor. *Psychopharmacologia*, **12**, 278–285.

VEDERNIKOV, Y. P. & AFRIKANOV, I. I. (1969). On the role of a central adrenergic mechanism in morphine analgesic action. *J. Pharm. Pharmac.*, **21**, 845–847.

VERRI, A. R., GRAEFF, F. G. & CORRADO, A. P. (1967). Antagonism of morphine analgesia by reserpine and alpha-methytyrosine and the role played by catecholamines in morphine analgesic action. *J. Pharm. Pharmac.*, **19**, 264–265.

WAY, E. L., LOH, H. H. & SHEN, F. (1968). Morphine tolerance, physical dependence and synthesis of brain 5-hydroxytryptamine. *Science*, **162**, 1290–1292.

WINTER, C. A. & FLATAKER, L. (1951). The effect of cortisone, desoxycorticosterone and adrenocorticotrophic hormone upon the responses of animals to analgesic drugs. *J. Pharmac. exp. Ther.*, **103**, 93–105.

WISE, C. D. & RUELIUS, H. W. (1968). The binding of serotonin in the brain: a study in vitro of physicochemical factors and drugs. *Biochem. Pharmac.*, **17**, 617–631.

YEN, H. C. Y., KATZ, M. H. & KROP, S. (1970). Effects of various drugs on 3,4-dihydroxyphenylalanine (DL-DOPA) induced excitation (aggressive behaviour) in mice. *Toxicol. Appl. Pharmac.*, **17**, 597–604.

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