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Effects of α-methyl-*p*-tyrosine, *p*-chlorophenylalanine, L-β-(3,4-dihydroxyphenyl)alanine, 5-hydroxytryptophan and diethyldithiocarbamate on the analgesic activity of morphine and methylamphetamine in the mouse

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Summary

1. The analgesic activity of sympathomimetic drugs does not appear to involve a peripheral component.

2. Drugs causing changes in morphine analgesia have similar effects on the analgesia produced by methylamphetamine.

3. The analgesia produced by morphine and methylamphetamine is increased by drugs which increase the ratio of brain 5-hydroxytryptamine (5-HT) to dopamine.

4. The analgesia is decreased by drugs causing a fall in brain 5-HT or a rise in dopamine relative to 5-HT.

Introduction

The demonstration of the analgesic activity of sympathomimetic drugs (Kiessig & Orzechowski, 1940) prompted many workers to investigate the possibility that adrenergic mechanisms were involved in the activity of the narcotic analgesics. Most of the work has centred around the effects of drugs, known to cause changes in brain concentration of biogenic amines, on the analgesic activity of morphine.

Reserpine, which causes general depletion of biogenic amines, antagonizes the analgesia produced by morphine in the mouse (Sigg, Caprio & Schneider, 1958; Medaković & Banic, 1964) as does disulphiram (Vedernikov & Afrikanov, 1969), which depletes brain noradrenaline. However, Contreras & Tamayo (1966) demonstrated that dopamine decreased the analgesic activity of morphine in the rat. The effects, on morphine analgesia, reported for drugs which deplete noradrenaline and dopamine without affecting 5-hydroxytryptamine (5-HT) are widely divergent.

α-Methyl-*m*-tyrosine decreases (Medaković & Banic, 1964) and has no effect on (Rudzik & Mennear, 1965) morphine analgesia in the mouse. Similarly α-methyl-*p*-tyrosine decreases (Verri, Graeff & Corrado, 1967) and has no effect on (Fennessy & Lee, 1970) morphine analgesia. Depletion of brain 5-HT using *p*-chlorophenylalanine abolishes (Tenen, 1968) or has no effect on (Fennessy & Lee, 1970) morphine analgesia. However, both 5-HT and 5-hydroxytryptophan enhance the analgesia produced by morphine (Sigg *et al.*, 1958; Mercier, Etzensperger & Mercier, 1959).

The following experiments were designed to compare the analgesia produced by morphine with that produced by sympathomimetic drugs in the presence of various drugs known to cause changes in brain concentration of noradrenaline, dopamine and 5-HT, and to determine whether a unified pattern exists for the effects of these drugs on the analgesic activity of either morphine or sympathomimetic drugs.

A preliminary account of this work was given at the British Pharmacological society meeting at Dublin on 8th July, 1970.

Methods

Male and female white mice (25–35 g) of the Alderly Park strain were used. Analgesia was determined using two testing methods. The hot plate reaction time test was used as described by Bousfield & Rees (1969). Reaction times were measured every 5 min for 30 min after injection of analgesic and thereafter at 15 min intervals until the reaction time was not significantly different from that of concurrently tested, vehicle pretreated, control mice.

Writhing was induced in mice by an intraperitoneal injection of 0·5% acetic acid and the number of writhes in the subsequent 20 min period was counted. Drug pretreatment times were chosen so that writhing was counted over the period of maximum analgesic activity.

Drugs

All drugs were injected intraperitoneally and doses are quoted as the salts. The drugs used were morphine sulphate (Evans Medical), methylamphetamine hydrochloride (Burroughs Wellcome), methylphenidate hydrochloride (Ciba 'Ritalin'), (+)-amphetamine sulphate (Sigma Chemical Co.), ephedrine hydrochloride (May & Baker), metaraminol bitartrate (Merck, Sharp and Dohme), aminophylline (United Chemists Association Ltd.), 5-HT (Koch-Light Laboratories), α-methyl-*p*-tyrosine (Sigma Chemical Co.), *p*-chlorophenylalanine (Koch-Light Laboratories), L-β-(3,4-dihydroxyphenyl)alanine (Koch-Light Laboratories), sodium diethyldithiocarbamate (Kodak), iproniazid (Roche 'Marsilid'), reserpine (B.D.H.), vasopressin (Parke-Davis 'Pitressin'), propranolol (I.C.I. 'Inderal') and phenoxybenzamine (Smith Kline & French Laboratories 'Dibenyline').

Reserpine was dissolved in a minimal quantity of glacial acetic acid and then diluted with distilled water (the final pH was 5·3); *p*-chlorophenylalanine was suspended in 1% sodium carboxymethylcellulose; α-methyl-*p*-tyrosine was dispersed in phosphate buffer pH 7·4 and sufficient sodium hydroxide added just to effect solution; L-β-(3,4-dihydroxyphenyl)alanine was dissolved in 0·1 N HCl and phenoxybenzamine was dissolved in a small quantity of propylene glycol and then diluted with distilled water. All other drugs were dissolved in sterile saline.

Results

Effect of sympathomimetics on hot plate reaction time and writhing response

All five sympathomimetics and morphine caused significant increases in hot plate reaction time and a significant decrease in the number of writhes. Dose-response relationships obtained by the two methods are shown in Figs. 1 and 2. An increase in hot plate reaction time over concurrent controls of more than 10 s could not be

obtained with any of the sympathomimetics. A probable reason for this is that high doses of the central stimulant drugs cause such excitement that judgement of the end point is unreliable. In subsequent experiments the lower analgesic doses of the sympathomimetics were used.

Molar potency ratios obtained by the two methods, taking morphine as 1, are shown in Table 1.

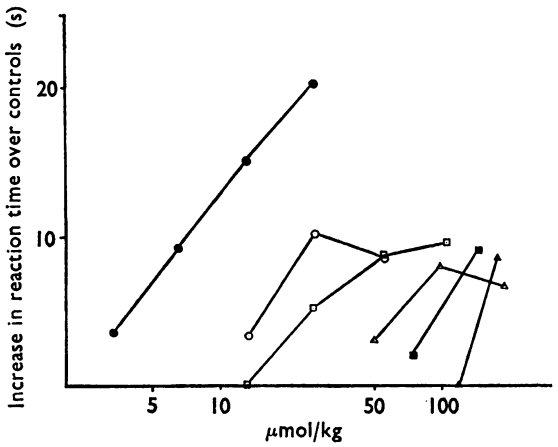

FIG. 1. Effect of intraperitoneal injection of morphine sulphate (●——●), amphetamine sulphate (○——○), methylamphetamine hydrochloride (□——□), ephedrine hydrochloride (△——△), methylphenidate hydrochloride (■——■) and metaraminol bitartrate (▲——▲) on the hot plate reaction time of the mouse. Reaction times are expressed as the maximum mean increase in reaction time over concurrent, saline-treated, control mice. Group sizes were six–twelve mice and a typical saline control group reaction time was 4·4±0·6 s for a group of nine mice.

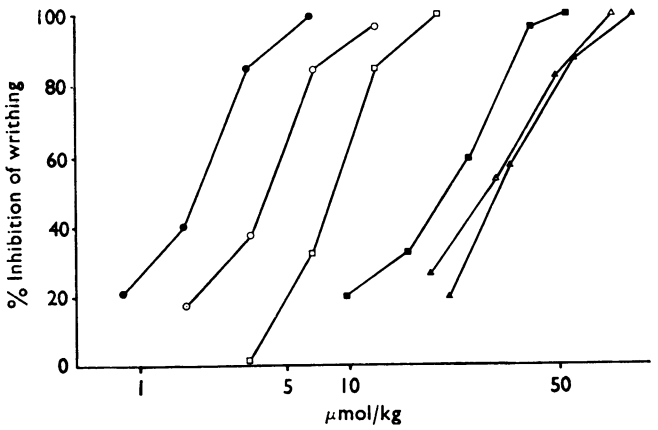

FIG. 2. Effect of morphine sulphate (●——●), amphetamine sulphate (○——○), methylamphetamine hydrochloride (□——□), methylphenidate hydrochloride (■——■), ephedrine hydrochloride (△——△) and metaraminol bitartrate (▲——▲) on the mouse writhing response to an intraperitoneal injection of 0·5% acetic acid in groups of six mice. The results are expressed as the percentage inhibition of writhing compared with control groups of mice treated with saline and 0·5% acetic acid.

Effect of vasopressor and vasodilator drugs on hot plate reaction time and the effects of α- and β-blocking drugs on the reaction times obtained in the presence of methylamphetamine

During the 60 min after injection of 10, 40 or 80 mg/kg aminophylline, mice showed hot plate reaction times not significantly different from saline control mice. Similar results were obtained with 0·08 and 0·8 units/kg vasopressin.

Neither pretreatment for 1 h with 5 mg/kg phenoxybenzamine (a dose which inhibited the piloerection produced by the sympathomimetics) nor pretreatment for 10 min with 10 mg/kg propranolol caused any changes in the reaction times obtained with mice injected with 10 mg/kg methylamphetamine. Furthermore, mixtures of 5 mg/kg phenoxybenzamine and 10 mg/kg propranolol, although making the mice extremely lethargic, induced no significant changes in the reaction time obtained with methylamphetamine.

Sodium diethyldithiocarbamate

Sodium diethyldithiocarbamate (400 mg/kg) reduced the reaction time induced by morphine and methylamphetamine when injected 1, 2, 4 and 6 h before the analgesic. However, the greatest reduction was obtained 2 and 4 h after injection of sodium diethyldithiocarbamate. In groups of six mice, the maximum mean reaction time (±S.E.) obtained with 10 mg/kg morphine sulphate after pretreatment for 4 h with sodium diethyldithiocarbamate was 7·0±0·9 s, whereas that obtained with concurrent morphine treated, control mice was 13·7±1·7 seconds. Similarly, mice pretreated with 400 mg/kg sodium diethyldithiocarbamate for 4 h and then injected with 10 mg/kg methylamphetamine had a maximum mean reaction time of 6·3±0·9 s, whilst control mice treated with methylamphetamine had reaction times of 11·3±0·9 seconds.

Mice treated with sodium diethyldithiocarbamate (400 mg/kg) alone had reaction times indistinguishable from saline control mice 1, 2, 4 and 6 h after injection.

*α-Methyl-*p*-tyrosine*

The effects of α-methyl-*p*-tyrosine on the reaction time induced by 10 mg/kg methylamphetamine hydrochloride and 10 mg/kg morphine sulphate are shown in Fig. 3.

α-Methyl-*p*-tyrosine caused a dose dependent rise in the reaction time produced by morphine and methylamphetamine, this being significant ($P<0·01$) with 150 mg/kg α-methyl-*p*-tyrosine. The figure shows the results obtained after pretreatment for 4 h with α-methyl-*p*-tyrosine; similar results were obtained after pretreatment for 1, 2 and 6 hours. When two doses of 100 mg/kg α-methyl-*p*-tyrosine were

TABLE 1. *Molar potency ratios, calculated from base rather than salt, obtained for morphine and five sympathomimetic drugs using the hot plate reaction time and writhing analgesic testing methods in mice*

Drug	Hot plate	Writhing test
Morphine	1	1
Amphetamine	0·70	0·92
Methylamphetamine	0·28	0·46
Ephedrine	0·14	0·08
Methylphenidate	0·12	0·12
Metaraminol	0·072	0·064

administered 8 and 4 h before 10 mg/kg morphine, a similar result was obtained. The maximum mean reaction time of a group of twelve mice was 28·0±3·1 s compared with morphine treated control mice, 19·2±2·6 seconds.

Mice treated with α-methyl-*p*-tyrosine alone showed a significant ($P<0·05$) increase in reaction time over saline control mice, 90–120 min after injection, but this effect was not apparent 4 h after injection.

When iproniazid (200 mg/kg) was injected 2 h before the analgesic and 2 h after the α-methyl-*p*-tyrosine a further increase in the reaction time induced by methylamphetamine and morphine occurred. In the case of morphine, 80% of the mice had reaction time exceeding the arbitrary cut off time of 45 seconds. The dose of iproniazid used had no effect on hot plate reaction times obtained after either saline, morphine or methylamphetamine injection.

L-β-(3,4-dihydroxyphenyl)alanine (L-dopa)

Figure 4 shows the results obtained when 200 mg/kg L-β-(3,4-dihydroxyphenyl)-alanine was injected into mice. Up to 40 min after injection L-β-(3,4-dihydroxyphenyl)alanine caused a significant ($P<0·001$) fall in the reaction times of mice compared with saline treated, control mice. When L-β-(3,4-dihydroxyphenyl)alanine was injected 30 min before morphine or methylamphetamine the reaction times obtained were not significantly different from saline treated, control mice. Furthermore, the reaction times of mice pretreated with 150 mg/kg α-methyl-*p*-tyrosine for 4 h and then given L-β-(3,4-dihydroxyphenyl)alanine 30 min before morphine or methylamphetamine could not be distinguished from saline treated control mice.

Pretreatment for 15 min with 200 mg/kg L-β-(3,4-dihydroxyphenyl)alanine partially inhibited the effects of morphine and methylamphetamine, the degree of inhibition increasing with time after injection.

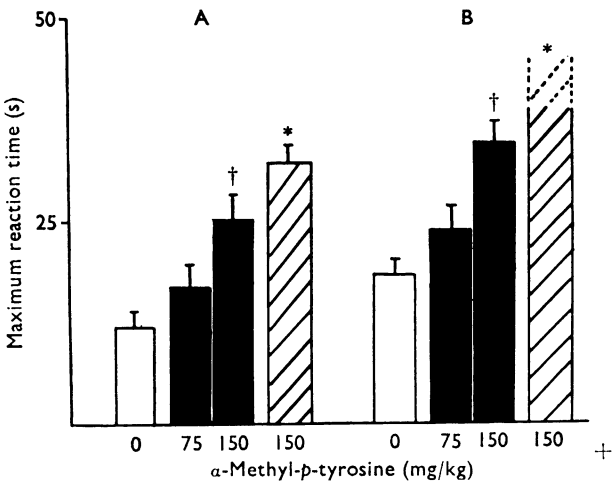

FIG. 3. Effects of methylamphetamine hydrochloride (10 mg/kg) (A) and morphine sulphate (10 mg/kg) (B) in saline control mice □ ($n=6$) and in mice pretreated with 75 and 150 mg/kg α-methyl-*p*-tyrosine ■ ($n=12$–18). ▨ A fourth group of mice, pretreated with 150 mg/kg α-methyl-*p*-tyrosine, had iproniazid injected 2 h before the analgesic ($n=12$). Results are expressed as maximum mean hot plate reaction times (±S.E.). Indicates that some reaction times were greater than the arbitrary cut off time of 45 seconds. *$P<0·05$; †$P<0·01$ when compared with corresponding morphine or methylamphetamine controls.

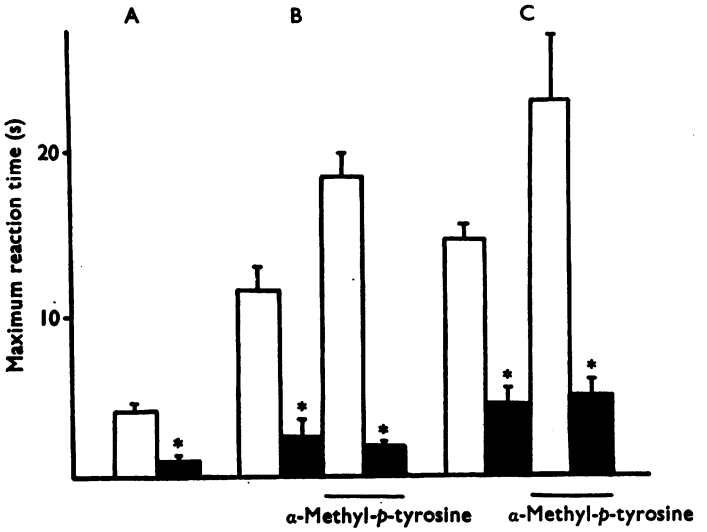

FIG. 4. Effect of L-β-(3,4-dihydroxyphenyl)alanine (L-dopa) on hot plate reaction times obtained in mice injected with saline, methylamphetamine and morphine. Control responses are shown for saline alone (A) and for methylamphetamine hydrochloride (10 mg/kg) (B) and morphine sulphate (10 mg/kg) (C) in mice pretreated with saline or 150 mg/kg α-methyl-*p*-tyrosine for 4 h □. The effects of 200 mg/kg L-β-(3,4-dihydroxyphenyl)alanine injected 30 min before the analgesic in similarly treated animals are shown ■ (n=6–8). Results are expressed as maximum mean reaction times (±S.E.). *P<0·001 when compared with corresponding control.

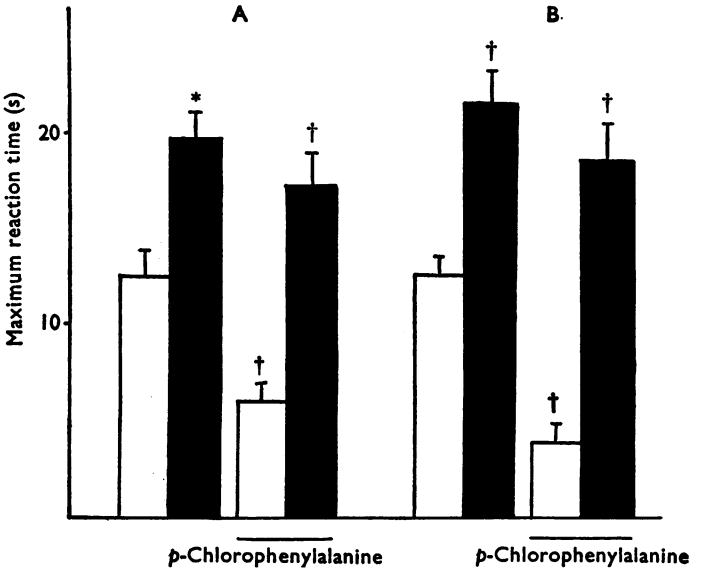

FIG. 5. Effect of methylamphetamine hydrochloride (10 mg/kg) (A) and morphine sulphate (10 mg/kg) (B) in control mice (n=6–9) and in mice pretreated with 150 mg/kg *p*-chlorophenylalanine twice daily for 3 days (n=8–12) □. The effects of 75 mg/kg 5-hydroxytryptophan, injected 10 min before the analgesic in similarly treated animals are shown ■ (n=6–12). Results are expressed as maximum mean hot plate reaction times (±S.E.). *P<0·01; †P<0·001 when compared with corresponding controls.

p-*Chlorophenylalanine*

Figure 5 shows the effects of 150 mg/kg *p*-chlorophenylalanine, twice daily for 3 days, on the hot plate reaction time of mice treated with 10 mg/kg morphine sulphate or 10 mg/kg methylamphetamine hydrochloride. *p*-Chlorophenylalanine abolished the increase in reaction time induced in control animals by the two drugs. Similar results were obtained with 100 mg/kg *p*-chlorophenylalanine daily for 3 days.

Figure 5 also shows the effects of 5-hydroxytryptophan (75 mg/kg) on the reaction time obtained with morphine or methylamphetamine alone and after pretreatment with *p*-chlorophenylalanine. The 5-hydroxytryptophan was injected 10 min before the analgesic, at which time its side effects, principally diarrhoea, were apparent. 5-Hydroxytryptophan significantly increased the reaction times of mice treated with morphine or methylamphetamine in the presence and absence of *p*-chlorophenylalanine.

Neither *p*-chlorophenylalanine nor 5-hydroxytryptophan had any significant effects on reaction times in the absence of the analgesics.

Effects of 5-hydroxytryptophan and L-*β-(3,4-dihydroxyphenyl)alanine on the actions of morphine and methylamphetamine in the reserpinized mouse*

Using the same doses and pretreatment times as before the effects of 5-hydroxytryptophan and L-β-(3,4-dihydroxyphenyl)alanine in the reserpinized mouse are shown in Table 2. Reserpine (5 mg/kg) abolished the increase in hot plate reaction time induced by morphine and methylamphetamine. The results shown in Table 2 were obtained after pretreatment for 4 h with reserpine, but similar results were obtained after pretreatment for 1, 2 and 24 h with reserpine. Reserpine alone only caused a significant ($P<0.05$) increase in reaction time 1 h after injection.

L-β-(3,4-dihydroxyphenyl)alanine did not modify the action of reserpine on the reaction times of mice treated with morphine and methylamphetamine, but 5-hydroxytryptophan antazonized its actions and, in the case of morphine, significantly ($P<0.005$) increased the reaction time above that produced by morphine alone.

Discussion

In view of the variety of peripheral actions exhibited by the sympathomimetic drugs, some experiments were carried out to determine whether the analgesia observed experimentally had a peripheral component. Intraperitoneal injection of

TABLE 2. *Effect of* L-*β-(3,4-dihydroxyphenyl)alanine and 5-hydroxytryptophan on the hot plate reaction times obtained with methylamphetamine and morphine in the reserpinized mouse*

Treatment	Methylamphetamine	Morphine
Control	11·4±0·4 s	12·8±1·4 s
Reserpine ((5 mg/kg)/4 h)	5·4±0·7 s*	3·9±0·6 s*
Reserpine+L-β-(3,4-dihydroxyphenyl)alanine ((200 mg/kg)/30 min)	4·5±1·1 s*	3·1±0·6 s*
Reserpine+5-hydroxytryptophan ((75 mg/kg)/10 min)	13·9±0·7 s	30·5±4·4 s†

All values are the maximum mean ±S.E.M. of six–twelve mice and refer to hot plate reaction times obtained with 10 mg/kg morphine or methylamphetamine. *$P<0.001$; †$P<0.005$ when compared with corresponding control. Reserpine (5 mg/kg) was administered 4 h before the analgesic, L-β-(3,4-dihydroxyphenyl)alanine (200 mg/kg) 30 min before and 5-hydroxytryptophan (75 mg/kg) 10 min before the analgesic.

non-specific vasoconstrictor and vasodilator drugs caused no change in the reaction time of mice, compared with saline control mice. The increase in reaction time induced by methylamphetamine was uneffected by propranolol or phenoxybenzamine, indicating that peripheral α- and β-receptors are not involved in the analgesia. Furthermore, Madinaveitia (personal communication) has demonstrated that local infusion of adrenaline into the mouse paw decreased the temperature at which the mouse responded to a heat stimulus (an effect tending to decrease hot plate reaction time). Thus it appears unlikely that the analgesia exhibited by the sympathomimetics contains a peripheral component. A similar conclusion was reached by Colville & Chaplin (1964) using the rat inflamed foot as the analgesic testing method. Diminution of the analgesic activity of morphine by propranolol has been reported in the mouse (Heller, Saavedra & Fischer, 1968), but Fennessy & Lee (1970) were unable to confirm this observation.

Diethyldithiocarbamate reduced the reaction time obtained with morphine and methylamphetamine. This drug, a dopamine β-hydroxylase inhibitor, causes a fall in noradrenaline content and a rise in dopamine in peripheral tissues of the rat and rabbit (Collins & West, 1968) and although its actions in the brain are more complicated, Carlsson, Lindqvist, Füxe & Hokfelt (1966) demonstrated a general fall in brain noradrenaline and a rise in dopamine in the brain stems and hemispheres of the rat. Increases in reaction time were obtained with mice pretreated with α-methyl-*p*-tyrosine in the presence of morphine and methylamphetamine. α-Methyl-*p*-tyrosine, a tyrosine hydroxylase inhibitor, also causes a fall in brain noradrenaline; however, there is a concurrent fall in brain dopamine (Spector, 1966; Weissman & Koe, 1965). Depletion of noradrenaline and dopamine is associated with a marked reduction in activity, which could increase hot plate reaction time, but the reaction times of mice treated with α-methyl-*p*-tyrosine and no analgesic were not significantly different from saline control mice. Furthermore, reserpine also depletes noradrenaline and dopamine, and reaction times induced by analgesics were reduced. Thus an increase in the reaction time produced by morphine and methylamphetamine appears to be associated with a fall in brain dopamine and decrease in reaction time is associated with a rise in dopamine in the mouse. The effect of L-β-(3,4-dihydroxyphenyl)alanine on analgesic activity shows the same pattern. There is a rise in brain dopa and dopamine, the analgesic activity of morphine and methylamphetamine is abolished and the actions of α-methyl-*p*-tyrosine on analgesic activity is reversed. The action of L-β-(3,4-dihydroxyphenyl)alanine in control animals may indicate that dopa or dopamine is involved in the maintenance of the pain threshold level in the untreated mouse.

Diethyldithiocarbamate, α-methyl-*p*-tyrosine and L-β-(3,4-dihydroxyphenyl)alanine cause no changes in brain content of 5-HT; however, drugs affecting 5-HT also modify analgesic activity. The increase in reaction time obtained with morphine and methylamphetamine is abolished by *p*-chlorophenylalanine, a tryptophan hydroxylase inhibitor, which lowers brain 5-HT content with little effect on noradrenaline and dopamine (Koe & Weissman, 1966; Somerville & Whittle, 1967). 5-Hydroxytryptophan, in both control animals and animals pretreated with *p*-chlorophenylalanine, raises brain 5-HT (Koe & Weissman, 1966) and increases the reaction time obtained with morphine and methylamphetamine. Thus, on the tryptaminergic side, analgesic activity is increased by a rise in 5-HT, but decreased by a fall.

Reserpine depletes noradrenaline, dopamine and 5-hydroxytryptamine from the brain and it abolishes the increase in reaction time produced by both morphine and methylamphetamine. The reversal of this effect is brought about by 5-hydroxytryptophan and not L-β-(3,4-dihydroxyphenyl)alanine, suggesting that the decrease in 5-HT content was responsible for the fall in reaction time induced by morphine and methylamphetamine in the presence of reserpine. This fits in with the pattern described above; however, brain dopamine content has fallen and in previous experiments this was associated with an increase in the analgesic activity of morphine and methylamphetamine. Thus it seems that increase in analgesic activity is not associated with the actual amounts of the various brain amines but with their relative amounts. Reaction times produced by morphine and methylamphetamine are increased in situations where dopamine falls relative to 5-HT and decreased in situations where either 5-HT falls or dopamine increases relative to 5-HT.

Morphine causes a fall in brain noradrenaline content (Vogt, 1954) and (+)-amphetamine causes a rise in 5-HT and a fall in noradrenaline and dopamine (Smith, 1965). Sparkes & Spencer (1969) have demonstrated that intraventricular injections of noradrenaline into rat brain antagonize morphine analgesia, whilst 5-HT prolongs it.

The action of iproniazid on the combined effects of α-methyl-*p*-tyrosine and the analgesics, morphine and methylamphetamine, may be ascribed to a rise in brain 5-HT content by virtue of monoamineoxidase inhibition. Another mechanism might be that the depressant effects of iproniazid on brain and liver enzymes cause a decreased metabolism of the analgesics. However, Rogers & Thornton (1968) investigated the increased toxicity of morphine in the presence of iproniazid in mice and found no correlation between increase in toxicity and inhibition of enzyme systems. Furthermore, blood concentrations of morphine were not significantly altered by the addition of iproniazid and finally they did show a correlation between rise in brain 5-HT with iproniazid and increased toxicity of morphine.

Many situations where 5-HT and catecholamines appear to be antagonistic have been described in the literature. 5-HT and noradrenaline have the opposite effects on the electrical activity of the brain (Monnier & Tissot, 1958) and on temperature regulation (Feldberg & Meyers, 1963). Catecholamines inhibit the enhanced effects of barbiturates induced by 5-HT and prevent tremors following intracisternal 5-HT (Garattini & Valzelli, 1965).

The evidence suggests that the analgesia produced by morphine and methylamphetamine is another situation where 5-HT and catecholamines are antagonistic.

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