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On the role of central nervous system catecholamines and 5-hydroxytryptamine in the nialamide-induced behavioural syndrome

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Summary

1. Intraperitoneal administration of nialamide, 200 mg/kg, to mice elicited a pronounced increase in motor activity and rectal temperature concomitant with a gradual increase in the concentrations of 5-hydroxytryptamine (5-HT), noradrenaline (NA) and dopamine in the brain.

2. In mice treated with L-tryptophan, 300 mg/kg, 1 h before nialamide, the increase in motor activity appeared earlier than after nialamide alone, and the hyperthermia was more pronounced. The increase in 5-HT concentrations in the brain was more pronounced in these animals, whereas the concentrations of NA and dopamine were of the same magnitude as after nialamide alone.

3. Treatment with *p*-chlorophenylalanine methylester-HCl (PCPA), 400 mg/kg, 24 h before nialamide partially antagonized the increase in motor activity and the accumulation of NA and dopamine was not significantly different from that observed after nialamide alone.

4. Treatment with PCPA, 800 mg/kg, 72, 48 and 24 h before nialamide, completely antagonized the increase in motor activity and rectal temperature. The accumulation of brain 5-HT was greatly depressed in these animals. The concentrations of dopamine 1, 3 and 6 h and the concentration of NA 6 h after the nialamide injection were significantly lower in the mice given PCPA 800 mg/kg × 3, than in the mice given nialamide alone.

5. Administration of DL-5-hydroxytryptophan, 30 mg/kg, 1 h after the nialamide injection, to mice pretreated with PCPA, 800 mg/kg × 3, restored the increase in motor activity and rectal temperature.

6. L-Tryptophan, 300 mg/kg, given 1 h before nialamide to mice pretreated with PCPA, 800 mg/kg × 3, elicited a moderate increase in motor activity and a slight increase in the accumulation of 5-HT in the brain when compared to that after PCPA, 800 mg/kg × 3, and nialamide.

7. Administration of α-methyltyrosine methylester, 200 mg/kg, 2 h before nialamide partially antagonized the increase in motor activity and completely antagonized the increase in rectal temperature elicited by nialamide alone. The accumulation of brain NA and dopamine was inhibited in these animals.

8. It is concluded that the excitation in mice, elicited by nialamide, is mediated largely via brain 5-HT, but that also the brain catecholamines seem to contribute to this effect.

Introduction

Monoamine oxidase (MAO) inhibitors generally increase endogenous monoamine concentrations and the concentrations of monoamines formed from exogenous precursors. Among the pharmacological properties they apparently share is psychomotor stimulation (for review see Pletscher, 1966), and it seems likely that there is a causal relation between these biochemical and behavioural effects.

In analysing the respective roles of the catecholamines (CA) and the 5-hydroxytryptamine (5-HT) accumulated in the brain after MAO-inhibition, several possibilities can be considered. Corrodi (1966) reported that the nialamide syndrome in mice was antagonized by pretreatment with drugs which block the accumulation of both 5-HT and CA (i.e., α-*n*-propyl-3,4-dihydroxyphenylacetamide or L-α-methyldopa) but not by pretreatment with the methylester of DL-α-methyltyrosine (α-MT), an inhibitor of the CA-synthesis which selectively blocks the accumulation of CA. This author suggested that the 5-HT accumulation after nialamide is of predominant importance for the behavioural effects.

More recently, *p*-chlorophenylalanine, a drug that more selectively depletes 5-HT (Koe & Weissman, 1966) has become available. This drug inhibits the tryptophan hydroxylase activity *in vivo* in the brain (Jéquier, Lovenberg & Sjoerdsma, 1967; see also Koe, 1971). Both increased and decreased motor activity have been reported after treatment with *p*-chlorophenylalanine (for references, see **Discussion**). At present no correlation has been established between the biochemical and the behavioural effects mentioned.

In the present study mice were pretreated with *p*-chlorophenylalanine or, in one experiment, α-MT, before the administration of nialamide. Pretreatment with *p*-chlorophenylalanine antagonized both the 5-HT accumulation in the brain and the behavioural effects seen after nialamide. Further investigations were conducted to determine whether the 5-HT precursors L-tryptophan or DL-5-hydroxytryptophan (5-HTP) could restore the nialamide syndrome. The effects of the various treatments on motor activity and brain monoamine concentrations were analysed.

Methods

The experiments were performed on male mice weighing about 20 g, strain N.M.R.I., at an environmental temperature of 24°–28° C. All injections were intraperitoneal.

A. *Motor activity.* The motor activity was measured by means of Animex activity meters (Svensson & Thieme, 1969) on groups of three mice. Five minutes after the administration of nialamide-HCl (200 mg/kg, 10 ml/kg) or NaCl solution (0·9% w/v, same volume), the animals were put into the test cages, and their activity was recorded in 10 min periods for 6·5 hours. Some of the mice were pretreated with *p*-chlorophenylalanine methylester–HCl (PCPA), 400 mg/kg 24 h, or 3 injections of 800 mg/kg (800 mg/kg × 3) 72, 48 and 24 h before the injection of nialamide or 0·9% w/v NaCl solutions (saline).

L-Tryptophan, 300 mg/kg, was given, 1 h before the injection of nialamide to some of the animals treated with PCPA, 800 mg/kg × 3, and nialamide or with nialamide alone. Other mice receiving PCPA, 800 mg/kg × 3, and nialamide, were injected with 5-HTP, 300 mg/kg, 1 h after nialamide. α-MT, 200 mg/kg, was administered 2 h before nialamide in some experiments.

B. *Biochemical experiments.* Analyses of the concentrations of noradrenaline (NA), dopamine and 5-HT in the brain were performed on animals treated as above. The analyses were performed 0, 1, 3 and 6 h after the nialamide injection. The animals used for biochemical studies were not tested for activity or rectal temperature. The brains of four mice were pooled for the determinations of CA or 5-HT. Noradrenaline was determined according to Bertler, Carlsson & Rosengren (1958), dopamine according to Carlsson & Waldeck (1958), as modified by Carlsson & Lindqvist (1962), and 5-HT according to Bertler (1961), as modified by Andén & Magnusson (1967).

C. *Temperature measurements.* In other animals, treated as above, the rectal temperature was measured by means of a telethermometer (model 43 TK, Yellow Springs Instrument Co., Inc.).

D. *Statistics.* The results were analysed using analysis of variance (with one criterion of classification) followed by t test, or, in case of CA accumulation after nialamide, the determination of confidence intervals (Scheffé, 1959). For the motor activity measurements, the difference between the various experimental groups was calculated for every 20 min period.

A probability level of $P<0{\cdot}05$ was considered to show significant difference for all comparisons made.

Results

A. *Motor activity.* The results of the motor activity measurements are demonstrated in Figures 1A and B. Nialamide elicited an initial depression of motor activity lasting 80 min, followed by an increase in activity that was significant, compared with saline-treated controls, from 140 min (time intervals refer to time after initiation of activity measurements).

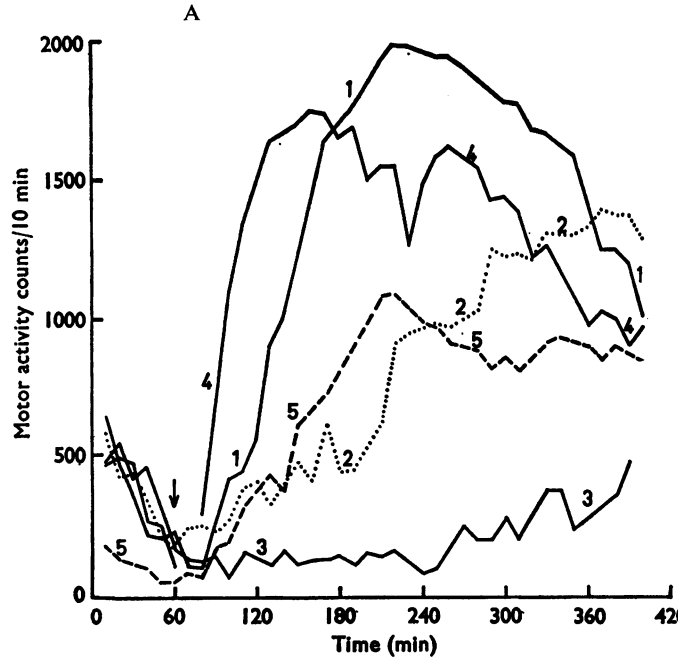
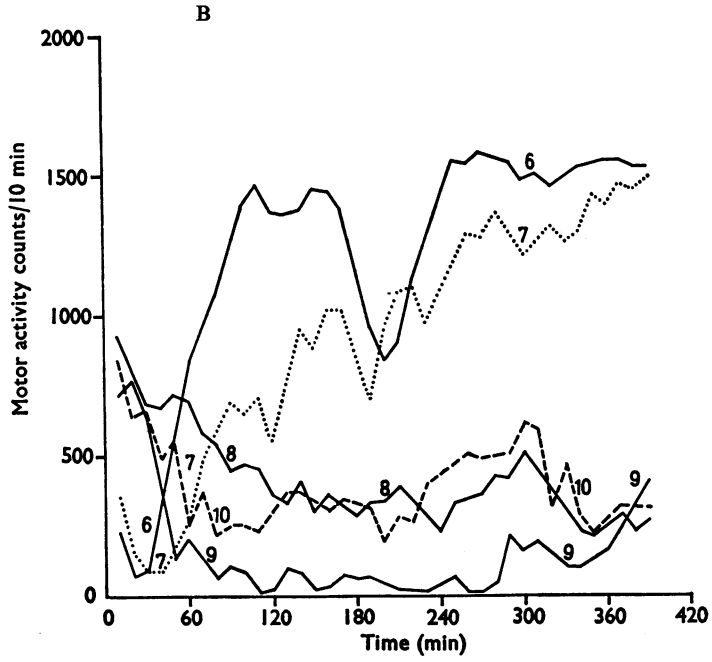


FIG. 1A (see p. 35).



FIGS. 1A and B. Effects of pretreatment with different amine synthesis inhibitors and precursor substances upon the hyperactivity caused by nialamide. Motor activity was recorded for successive 10 min periods in groups of 3 mice. Each point represents the mean activity of n groups. The recording of activity was started 5 min after the injection of nialamide, 200 mg/kg, or isotonic NaCl solution (controls). All injections were given i.p. The differences in motor activity between the various experimental groups were calculated at every 20 min period, by means of analysis of variance (with one criterion of classification) followed by t test. Statistics: Variance within groups at some of these time intervals are presented below. All F-values were significant.

	Minutes after the start of activity measurements					
	60	120	180	240	300	360
Variance within group	33225	137014	117766	97431	113150	88413

FIG. 1A. 1. Nialamide, $n=5$. 2. PCPA 400 mg/kg 24 h before nialamide, $n=5$. 3. PCPA, 800 mg/kg, 72, 48 and 24 h before nialamide, $n=6$. 4. PCPA, 800 mg/kg, 72, 48 and 24 h before and DL-5-HTP, 30 mg/kg 1 h after (arrow) nialamide, $n=4$. 5. α-MT, 200 mg/kg, 2 h before nialamide, $n=4$. Missing part of line: The recording of activity was interrupted for 10 min for injection of DL-5-HTP.

FIG. 1B. 6. L-Tryptophan, 300 mg/kg, 1 h before nialamide, $n=4$. 7. PCPA, 800 mg/kg, 72, 48 and 24 h before and L-tryptophan, 300 mg/kg, 1 h before nialamide, $n=7$. 8. Saline, $n=7$. 9. PCPA, 400 mg/kg, 24 h before saline, $n=4$. 10. PCPA, 800 mg/kg, 72, 48 and 24 h before saline, $n=4$.

The activity apparently reached a maximum after about 4 hours. Between 2 and 4 h the mice showed a continuously increased excitation and restlessness but no apparent aggressiveness. At the beginning of this period rapid head movements appeared and later tremor developed. These effects were more pronounced after 4 h, particularly the tremor, and hyperextension and abduction of the hind legs also appeared. These motor disturbances largely impaired the coordinated movements, especially at the end of the recording period when tremor and jumping predominated. During the period of increasing motor activity, the registered counts reflected mainly coordinated locomotion. Later, and especially at the end of the recording period, the counts, to a large extent, reflected uncoordinated movements.

In mice treated with L-tryptophan and nialamide both the initial depression and

the excitation appeared earlier than after nialamide alone: the activity in the animals given L-tryptophan was significantly lower during the first 20 min and higher between 60 and 120 minutes. Between 180 and 240 min the activity was, however, significantly lower than that of mice given nialamide alone. Thereafter the motor activity of the two groups did not differ.

After treatment with PCPA, 400 mg/kg, and nialamide an initial depression of motor activity appeared which was similar to that after nialamide alone. This depression was followed by a gradually increased activity which was significant in comparison with that of saline-treated animals at and after 220 minutes. The increase in activity was, however, less pronounced than after nialamide alone; the difference between the groups showing significance between 140 min and 320 minutes. Qualitatively the behavioural changes were similar to those elicited by nialamide alone, but they were less pronounced and developed later. The uncoordinated movements were especially reduced.

In mice pretreated with PCPA, 800 mg/kg × 3, nialamide also produced an initial depression of motor activity. Thereafter the activity did not rise, and it was not significantly different from that obtained with saline-treated controls. As assessed by gross observation, the behaviour of the experimental group did not differ from that of the mice treated with saline. The motor activity of the animals was lower than after nialamide alone and also lower than after PCPA, 400 mg/kg, plus nialamide. The differences were significant after 140 min and 220 min, respectively.

5-HTP given to mice pretreated with PCPA, 800 mg/kg × 3, and nialamide elicited an immediate increase in motor activity, reaching a maximum after about 1 h and lasting for several hours. The activity was significantly increased when compared with that of mice given PCPA, 800 mg/kg × 3, and nialamide from 40 min after the 5-HTP injection. Grossly, the behaviour of the animals was similar to that of mice treated with nialamide alone. However, a few of the animals died at the end of the measurement period.

Some mice pretreated with PCPA, 800 mg/kg × 3, received L-tryptophan, 300 mg/kg, 1 h before nialamide. After an initial decrease, they displayed an increase in motor activity. This increase was smaller than that of mice given nialamide alone between 180 and 300 min, but it was larger than that of animals given PCPA, 800 mg/kg × 3, and nialamide from 80 minutes.

PCPA alone had only a slight effect on activity. When compared with controls treated with saline a minor, although significant reduction in activity was found after PCPA, 400 mg/kg, between 40 and 100 min, and after PCPA, 800 mg/kg × 3, between 60 and 80 minutes. No changes in gross behaviour could be detected.

In mice pretreated with α-MT and given nialamide, motor activity was significantly lower between 140 and 360 min than that of mice given nialamide alone. When compared with controls given saline the activity was initially depressed but increased from 180 min after the nialamide injection.

B. *Biochemical experiments.* The results of the biochemical determinations are shown in Tables 1A, B and C. Nialamide alone produced a gradual increase in central 5-HT and CA concentrations. The increase in 5-HT and dopamine was significant from 1 h and the increase in NA significant from 3 h after nialamide. The increase in 5-HT was more pronounced than that in CA. In mice treated with L-tryptophan and nialamide, 5-HT was increased at all intervals studied when

TABLE 1. *Concentrations of 5-hydroxytryptamine and catecholamines in mouse brain after different drug treatments*

TABLE 1A.

	Treatment	5-hydroxytryptamine μg/g (Hours after nialamide) 0	1	3	6
A.	Nialamide	0·43±0·01 (4)	0·82±0·04 (4)	1·30±0·08 (4)	1·71±0·07 (4)
B.	L-Tryptophan, 300 mg/kg, 1 h before nialamide	0·55±0·02 (4)	1·34±0·10 (3)	1·78±0·03 (4)	—
C.	PCPA, 400 mg/kg, 24 h before nialamide	0·26±0·01 (4)	0·48±0·01 (4)	0·72±0·04 (4)	0·85±0·06 (4)
D.	PCPA, 800 mg/kg, 72, 48 and 24 h before nialamide	0·08±0·02 (3)	0·22±0·01 (3)	0·25±0·01 (4)	0·33±0·03 (4)
E.	PCPA, 800 mg/kg×3 (as in D) and L-tryptophan, 300 mg/kg, 1 h before nialamide	0·13±0·02 (4)	0·39±0·01 (4)	0·41±0·01 (4)	—

TABLE 1B.

	Treatment	Noradrenaline μg/g (Hours after nialamide) 0	1	3	6
A.	Nialamide	0·44±0·02 (8)	0·52±0·04 (4)	0·65±0·04 (4)	0·74±0·03 (4)
B.	L-Tryptophan, 300 mg/kg, 1 h before nialamide	0·39±0·04 (4)	0·55±0·02 (4)	0·68±0·02 (4)	—
C.	PCPA, 400 mg/kg, 24 h before nialamide	0·38±0·01 (4)	0·53±0·03 (4)	0·59±0·06 (4)	0·65±0·03 (4)
D.	PCPA, 800 mg/kg, 72, 48 and 24 h before nialamide	0·41±0·01 (9)	0·53±0·02 (7)	0·59±0·02 (10)	0·58±0·02 (10)
E.	PCPA, 800 mg/kg×3 (as in D) and L-tryptophan, 300 mg/kg, 1 h before nialamide	0·42±0·02 (4)	0·41±0·00 (3)	0·55±0·01 (4)	—
F.	α-MT, 200 mg/kg, 2 h before nialamide	0·31±0·02 (4)	0·26±0·02 (4)	0·29±0·02 (4)	0·28±0·06 (2)

TABLE 1C.

	Treatment	Dopamine μg/g (Hours after nialamide) 0	1	3	6
A.	Nialamide	0·96±0·07 (8)	1·46±0·18 (4)	1·74±0·27 (4)	1·78±0·24 (4)
B.	L-Tryptophan, 300 mg/kg, 1 h before nialamide	—	1·39±0·02 (4)	1·51±0·08 (4)	—
C.	PCPA, 400 mg/kg, 24 h before nialamide	1·01±0·16 (4)	1·31±0·19 (4)	1·72±0·40 (4)	1·57±0·28 (4)
D.	PCPA, 800 mg/kg, 72, 48 and 24 h before nialamide	0·81±0·04 (10)	0·93±0·03 (8)	1·09±0·04 (4)	1·11±0·05 (10)
E.	PCPA, 800 mg/kg×3 (as in D) and L-tryptophan, 300 mg/kg, 1 h before nialamide	0·61±0·02 (4)	0·81±0·02 (3)	0·92±0·04 (4)	—
F.	α-MT, 200 mg/kg, 2 h before nialamide	0·36±0·02 (4)	0·38±0·01 (4)	0·32±0·02 (4)	0·41±0·03 (4)

Concentrations of 5-hydroxytryptamine, noradrenaline, and dopamine in mouse brain at different time intervals after treatment with nialamide 200 mg/kg alone or in combination with different amine precursor substances or synthesis inhibitors. All drugs were given i.p. Each value represents the mean of (*n*) determinations ±S.E.M.

compared with results obtained with mice given nialamide only. CA-concentrations were, in contrast, the same in the two groups.

Treatment with PCPA, 400 mg/kg, induced a definite reduction in 5-HT, a slight but significant reduction in NA, but no change in dopamine. Injection of nialamide to mice pretreated with PCPA, 400 mg/kg, produced a gradual increase in central 5-HT, exceeding the concentrations in saline-treated controls at the 3 and 6 h intervals. However, the 5-HT concentrations at all intervals were lower than those in mice given nialamide alone. The increase in CA was not significantly different from that found after nialamide alone.

Treatment with PCPA, 800 mg/kg × 3, induced a pronounced depletion of 5-HT but no significant change in the CA concentrations. After the injection of nialamide to mice pretreated with PCPA, 800 mg/kg × 3, there was only a slight increase in the 5-HT concentrations. Even after 6 h the 5-HT concentration was below that in controls given saline. The dopamine concentrations were significantly lower in mice pretreated with PCPA, 800 mg/kg × 3, at 1, 3 and 6 h after the injection of nialamide, when compared to those, at the corresponding time intervals, after nialamide alone. The NA concentrations were significantly lower only at the 6 h interval. The accumulation of dopamine and NA in mice given only nialamide was calculated as the difference between the mean values at 0 and 6 h and was found to be 0·82 and 0·30 μg/g, respectively. In mice treated with PCPA, 800 mg/kg × 3, and nialamide, the corresponding accumulation of dopamine and NA was 0·30 and 0·17 μg/g, respectively. However, the two values for dopamine-accumulation as well as the values for NA-accumulation were not statistically different.

In mice pretreated with PCPA, 800 mg/kg × 3, the administration of L-tryptophan, 300 mg/kg, induced a slight but significant increase in the 5-HT concentrations, whereas the CA concentrations were not significantly changed. In mice pretreated with PCPA, 800 mg/kg × 3, and L-tryptophan, the 5-HT concentrations 1 and 3 h after nialamide were also slightly elevated, when compared with those in mice given PCPA, 800 mg/kg × 3, and nialamide. The increase in the CA concentration was of the same magnitude as after PCPA, 800 mg/kg × 3, and nialamide, except for a significantly lower NA concentration 1 h after nialamide.

Treatment with α-MT reduced brain CA concentrations. Nialamide produced no significant changes in CA concentrations after α-MT pretreatment.

C. *Temperature measurements.* The results of the temperature measurements are shown in Figures 2A and B. The rectal temperature was slightly reduced 1 h after the nialamide injection, but after 3 and 6 h it was increased. After L-tryptophan alone there was a tendency toward a reduction in temperature ($P<0·1$). One and 3 h after the injection of nialamide to mice pretreated with L-tryptophan, the temperature changes were similar to those induced by nialamide alone.

Pretreatment with PCPA, 800 mg/kg × 3, which by itself had no effect on the temperature, prevented the increase in temperature, measured 3 and 6 h after the injection of nialamide alone, but not the decrease at the 1 h interval. In mice pretreated with PCPA, 800 mg × 3, the injection of L-tryptophan produced a definite reduction in temperature. Thus, PCPA appeared to potentiate the hypothermic effect of L-tryptophan. After injection of nialamide to mice pretreated

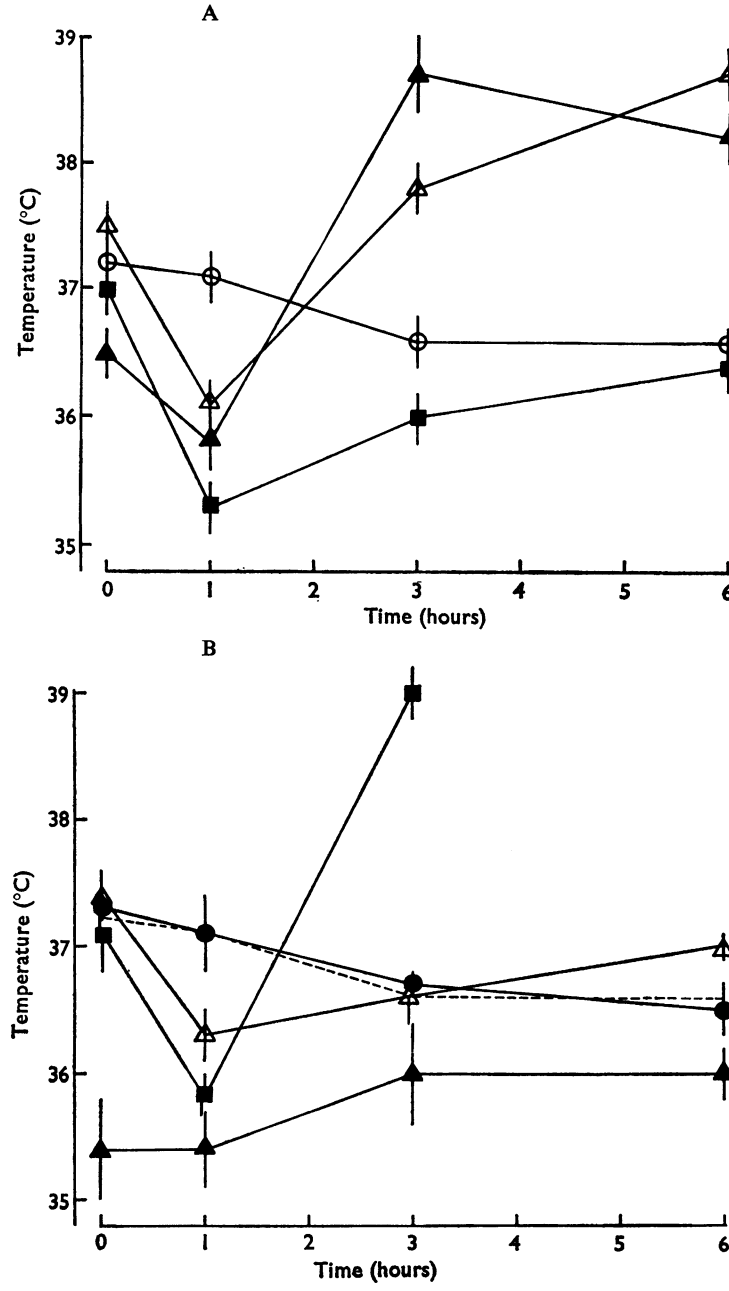


FIG. 2A and B. Rectal temperature in mice at different time intervals after treatment with nialamide, 200 mg/kg, alone or in combination with different precursor substances or synthesis inhibitors. Each point represents the mean temperature of 3 to 5 mice. Vertical bars represent S.E.M. All drugs were given i.p.

FIG. 2A. ○——○, Isotonic saline. △——△, Nialamide. ▲——▲, L-Tryptophan, 300 mg/kg, 1 h before nialamide. ■——■, α-MT, 200 mg/kg, 2 h before nialamide.

FIG. 2B. - - - - - Isotonic saline. △——△, PCPA, 800 mg/kg, 72, 48 and 24 h before nialamide. ■——■, PCPA, 800 mg/kg, 72, 48 and 24 h before and DL-5-HTP, 30 mg/kg, 1 h after nialamide. ▲——▲, PCPA, 800 mg/kg, 72, 48 and 24 h and L-tryptophan, 300 mg/kg 1 h before nialamide. ●——●, PCPA, 800 mg/kg, 72, 48, and 24 h before saline.

with PCPA, 800 mg/kg × 3, and L-tryptophan, the temperature remained at the same low level as before the nialamide injection.

Injection of 5-HTP 1 h after nialamide in mice pretreated with PCPA, 800 mg/kg × 3, produced a pronounced increase in temperature, when measured 2 h after 5-HTP. Five hours after the 5-HTP injection some of the animals had died.

Two hours after the administration of α-MT the rectal temperature was unchanged. One hour after the injection of nialamide to the α-MT pretreated mice, there was a significantly greater reduction in temperature than that, measured at the same time interval, after nialamide alone. After 3 and 6 h, the temperature in the α-MT plus nialamide-treated mice approximated that in the mice given saline.

Discussion

The behavioural and biochemical effects of nialamide found in the present investigation confirm earlier reports (see Rowe, Bloom, P'An & Finger, 1959; Carlsson, Lindqvist & Magnusson, 1960; Pletscher, 1966). Thus both endogenous CA and 5-HT concentrations were increased by nialamide, the increase in 5-HT being more pronounced. Moreover, a pronounced increase in motor activity and hyperthermia was observed, which was preceded 1 h after the injection of nialamide, by a depression of the motor activity and a concomitant hypothermia. These initial effects persisted after pretreatment both with PCPA and α-MT, drugs which effectively prevented the accumulation of 5-HT and CA, respectively. The effects observed can therefore not be related to the changes in the monoamine concentrations. In the periphery MAO inhibitors appear to diminish the NA release accompanying nerve stimulation (Hucović & Muscholl, 1962). Whether some mechanism, impairing the central transmission, pre- or postsynaptically, is involved in the initial behavioural depression produced by nialamide, remains to be elucidated. Other mechanisms, unrelated to monoaminergic transmission, may also be involved.

The large 5-HT depletion as well as the slight decrease in NA induced by PCPA also confirm earlier reports (Koe & Weissman, 1966; Welch & Welch, 1967). The slight reduction of motor activity obtained after PCPA alone, is in all probability not related to the degree of 5-HT depletion in the brain since the slight depression of motor activity was not significantly more pronounced after PCPA, 800 mg/kg × 3, than after 400 mg/kg of the synthesis inhibitor, whereas the 5-HT depletion was significantly greater after the larger dose. This view is in agreement with that of Volicer (1969). Both an increased (Pirch, 1969; Fibiger & Campbell, 1971), and a decreased motor activity (Tenen, 1967; Volicer, 1969) is reported in mice and rats depleted of 5-HT by *p*-chlorophenylalanine.

The ability of PCPA to prevent the nialamide-induced behavioural effects and the 5-HT accumulation was apparently dose-dependent.

The formation of 5-HT from exogenously administered 5-HTP is largely unaffected by PCPA (Koe & Weissman, 1966). Administration of 5-HTP, 30 mg/kg, restored the typical picture of nialamide induced excitation in mice pretreated with PCPA, 800 mg/kg × 3. It has been shown that 5-HT, formed in the brain after the administration of large doses of 5-HTP, causes displacement of endogenous CA (Fuxe, Butcher & Engel, 1971). However, since lower doses appear to cause 5-HT accumulation predominantly in 5-HT neurones, it seems reasonable to

assume that the restored excitation after 5-HTP was due largely to an increased formation of transmitter substance in the 5-HT neurones.

It may be concluded that the antagonizing effect of PCPA on the nialamide syndrome is largely due to inhibition of the 5-HT synthesis. However, the possibility cannot be excluded that the CA are involved to some extent in the action of PCPA.

Pretreatment with PCPA, 800 mg/kg × 3, also induced a reduction in the dopamine concentrations 1, 3 and 6 h, and the NA concentrations 6 h after nialamide, when compared at corresponding time intervals to the concentrations after nialamide alone. This could be due either to an increased release or an impaired synthesis of the CA. An increased release of the CA, induced by PCPA, seems unlikely since the CA concentrations were only slightly affected by treatment with PCPA alone. Possibly a metabolite of PCPA, e.g., *p*-chlorophenylethylamine (Koe & Weissman, 1966), the concentration of which is probably increased after inhibition of MAO, might cause displacement of the CA. However, the lack of stimulation or sympathomimetic signs in the mice treated with PCPA, 800 mg/kg × 3, and nialamide, argues against such a mechanism. PCPA has been reported to produce a slight and relatively short-lasting inhibition of tyrosine hydroxylase *in vivo* (Koe & Weissman, 1966; McGeer, Peters & McGeer, 1968). Since treatment with PCPA, 800 mg/kg × 3, alone did not significantly reduce the levels of the CA, a direct synthesis inhibition seems unlikely. Possibly PCPA might, after MAO inhibition, exert its effect on the brain CA through more indirect mechanisms. For example, the accumulation and increased release of 5-HT produced by nialamide which is inhibited by pretreatment with PCPA, may induce an increased CA synthesis. Persson (1970) reported that lysergic acid diethylamide, which stimulates central 5-HT receptors (Andén, Corrodi, Fuxe & Hökfelt, 1968), produced an increased accumulation of ^{3}H-dopamine and ^{3}H-NA in the brains of rats given ^{3}H-tyrosine. This was interpreted as indicating a functional relation between central 5-HT and CA neurones. If so, PCPA may antagonize the nialamide-induced CA-accumulation indirectly, because of the absence of 5-HT receptor stimulation. The restoration by 5-HTP of the excitation in the PCPA plus nialamide pretreated mice, does not rule out the possibility that the reduced CA accumulation by PCPA in nialamide pretreated mice, may be of some functional significance.

Administration of L-tryptophan, before nialamide, accelerated the accumulation of 5-HT, whilst the accumulation of CA was unaffected. The latency for the behavioural effects was shortened. These observations with tryptophan lend support for the role of 5-HT in the nialamide syndrome and are in agreement with the findings in rats, recently reported by Grahame-Smith (1971) who used tranylcypromine as an MAO inhibitor.

L-Tryptophan given to mice treated with PCPA, 800 mg/kg × 3, plus nialamide induced a moderate excitation, whereas the levels of 5-HT only reached the values observed in controls treated with saline. Possibly the formation of tryptamine may contribute to this excitatory effect (cf. Hess & Doepfner, 1961). Andén, Corrodi & Fuxe (1971) have shown that tryptamine causes displacement of endogenous 5-HT and also directly stimulates central 5-HT receptors. However, Grahame-Smith (1971) found only very small amounts of tryptamine in the brains of rats given L-tryptophan, 1 g/kg, after pretreatment with the MAO inhibitor tranylcypromine.

Furthermore, the possibility cannot be excluded that the long-lasting depletion of brain 5-HT, produced by the pre-treatment with PCPA, may have induced a supersensitivity of the 5-HT receptors. If so, the moderate increase in 5-HT, reaching the levels in controls given saline, may have contributed, alone or in combination with tryptamine, to the observed increase in motor activity.

The ability of α-MT to partially prevent the nialamide-induced behavioural excitation, is in all probability due to inhibition of CA-synthesis. α-MT, alone, in this dose elicits depression of motor activity in mice (Svensson & Waldeck, 1971). Therefore, in the present study, the partial stimulation obtained by nialamide in the presence of α-MT seems likely to be due to excess of 5-HT.

Carlsson, Dahlström, Fuxe & Lindqvist (1965) reported that the excitation in rats, produced by a MAO inhibitor was much enhanced by exposing the animals to a high temperature (29° C). They also found, by a combined histochemical and biochemical approach, that after MAO inhibition, the release of CA and 5-HT was increased by exposing the animals to a high temperature. In the present study a large increase in temperature was observed in the groups of mice, which demonstrated the most pronounced excitation and where both the concentrations of CA and 5-HT in the brain were elevated. Probably this hyperthermia, by facilitating the release of the brain monoamines, contributed to the development of the pronounced excitation. It has been proposed that hypothalamic NA and 5-HT are involved in thermoregulation (Feldberg & Myers, 1963, 1964; for recent review see Lomax, 1970), and the nialamide induced hyperthermia may at least partially be mediated by effects of the drug on the temperature regulating centres. However, the increased motor activity, which accompanied the hyperthermia, may also have contributed to the temperature increase, by means of an increased heat production. Also, the drug treatments probably caused peripheral effects which may have affected the body temperature. The nialamide-induced hyperthermia seems to be dependent both on the accumulation of brain CA and 5-HT since it was antagonized by pretreatment with either α-MT or PCPA and, after the latter pretreatment, restored by the administration of 5-HTP.

The present observations suggest that the excitation in mice, elicited by nialamide, is highly dependent on the accumulation of 5-HT, since the excitation was antagonized in a dose-dependent manner by pretreatment with PCPA and restored after the administration of 5-HTP. Also the observation that pretreatment with L-tryptophan shortened the latency for the onset of the excitation after nialamide supports this view. However, no precise correlation was found between the concentrations of 5-HT and the registered motor activity. The lack of correlation could at least partly be due to differences in the levels of CA and tryptamine and to different receptor sensitivity. Also effects, unrelated to the monoamines, of the drugs utilized and differences in body temperature could contribute to the lack of correlation. Furthermore, the whole-brain concentrations of the transmitter substances do not necessarily reflect the amount available at the receptors, as pointed out by Grahame-Smith (1971).

The contribution of the CA in controlling motor activity is fairly well established (Hornykiewicz, 1966; Svensson & Waldeck, 1969; 1970; Svensson, 1971; for review see van Rossum, 1970). Administration of L-DOPA, but not 5-HTP, restores the gross behaviour and motor activity in animals pretreated with reserpine (Carlsson, Lindqvist & Magnusson, 1957). Although 5-HTP together with a peri-

pheral decarboxylase inhibitor can induce motor stimulation in mice (Modigh, 1972), this treatment has no effect in reserpine-pretreated mice (Modigh & Svensson, unpublished observations). Direct activation of central CA-receptors in reserpine-pretreated animals induces motor stimulation (Andén, Corrodi, Fuxe, Hökfelt, Hökfelt, Rydin & Svensson, 1970), and blockade of these receptors, e.g., by haloperidol, results in sedation. The accumulation of the CA, after nialamide, probably implies overflow of the amines onto the receptors (Carlsson *et al.*, 1960, 1965). This mechanism is likely to contribute to the increase in motor activity.

The role of 5-HT in the control of the motor activity is probably more indirect than that of the CA, and may be dependent on the functional state of the CA neurones. The fact that treatment with PCPA alone, which produced a large depletion of 5-HT, but had only a slight influence on the motor activity, and also the fact that the increase in motor activity was less pronounced in mice given α-MT before nialamide, are in agreement with this view. Further, the nialamide induced increase in motor activity is blocked by haloperidol (Modigh & Svensson, 1971, in preparation). Drugs stimulating central 5-HT receptors, e.g. lysergic acid diethylamide, as well as large doses of MAO-inhibitors elicit hallucinations in man. Possibly, an excessive stimulation of central 5-HT receptors induces similar sensory disturbances in animals, thereby producing excitation. Such an excitation may be reflected in the increased motor activity observed in the present study in response to nialamide treatment.

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