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DIFFERENT CENTRAL MEDIATION OF THE STIMULANT EFFECTS OF AMPHETAMINE AND ITS *p*-CHLORO ANALOGUE

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Abstract—After it had been shown that amphetamine and *p*-chloroamphetamine have a different influence on central monoamines, the former depleting noradrenaline, the latter 5-HT, the question of a different mode of action for the two compounds, being closely related in their pharmacological properties, was studied in mice using the locomotor effect evoked by both compounds. In the case of amphetamine, this could be suppressed by small doses of α-methyltyrosine as well as by higher doses of cyproheptadine for which an adrenolytic effect must be supposed, but it could not be suppressed by a depletion of the central 5-HT with *p*-chlorophenylalanine. This points to a purely adrenergic mediation of the effect of amphetamine. The locomotor effect of *p*-chloroamphetamine could be suppressed by depleting the central 5-HT with *p*-chlorophenylalanine, by low doses of the 5-HT antagonist cyproheptadine and by high doses of α-methyltyrosine. Thus, the *p*-chloro compound is dependent on 5-HT in order to exert its central stimulant effect, but an adrenergic link, supposedly lying behind the tryptaminergic one, is also involved.

IN A PREVIOUS study from our laboratory[1] it was shown that *p*-chloroamphetamine has the same qualitative pharmacodynamic properties as amphetamine: viz. central stimulant, anorexigenic and vasoconstrictor. The difference appeared to be a quantitative one only, the anorexigenic effect being more pronounced and the central stimulant as well as the vasoconstrictor effects being weaker than those of amphetamine. However, there is a striking difference in the central biochemical effects of both drugs: whereas amphetamine causes a depletion of noradrenaline and has no influence on the central 5-hydroxytryptamine (5-HT), *p*-chloroamphetamine as well as *p*-chloromethamphetamine were found to deplete the central 5-HT while being without influence on the central noradrenaline.[2,3] In our own experiments a significant depletion of 5-HT was found with as low a dose as 2 mg/kg D-*p*-chloroamphetamine orally in rats.

This biochemical difference between amphetamine and its *p*-chloro analogue in spite of qualitatively similar pharmacodynamical effects gave rise to the question whether these two compounds should depend on different mediators of their central action. The majority of investigators consider noradrenaline to be the mediator of the central effects of amphetamine.[4–10] Van Rossum and Hurkmans[11] believed dopamine to be the mediator of the locomotor effect of amphetamine while Ernst[12] believes dopamine to be the mediator of the gnawing compulsion caused by amphetamine. A role of 5-HT is discussed by Gelder and Vane[13] and by Stein.[6] Since

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amphetamine retains its central stimulant effect after depletion of the above mentioned central monoamines by reserpine, a direct effect on the central adrenergic receptor has been postulated.[14, 15]

In the present study the locomotor effect of amphetamine and *p*-chloroamphetamine was measured in mice and the effects of changing the central concentrations of noradrenaline and 5-HT as well as the effects of blocking the 5-HT receptors by cyproheptadine thereupon were studied. The results show that *p*-chloroamphetamine is dependent on 5-HT in order to display its stimulant effect whereas amphetamine is not.

MATERIAL AND METHODS

All experiments were done in mice of both sexes of the Leo strain with an average weight of 25 g.

(a) *Locomotor activity*. Groups of 5 mice were put into an activity cage of 28 × 48 cm and the interruptions of four parallel light beams were registered over periods of 10 min. At least 3 experiments were done at each dose level. The geometrical means are given in the figures and in Table 4.

(1) In the cyproheptadine series three groups of mice were tested in the box until the number of interruptions within 10 min had fallen to below 100 (usually after 3 courses). Then the control group received 0·1 ml water both i.p. and orally. The second group (positive controls) received 0·1 ml water i.p. together with an oral dose of, respectively, 2 mg/kg D-amphetamine or 4 mg/kg D-*p*-chloroamphetamine (calculated as the base). The third group received doses between 0·06 and 1 mg/kg cyproheptadine i.p. and one of the amphetamines in the dose mentioned orally. The volume of the whole medication never exceeded 10 ml/kg. 30 Min, 1 hr and 2 hr after the application, the locomotor activity was measured for periods of 10 min. The central stimulant effect was expressed as the sum of interruptions in the three periods corrected for the activity of the control group. The inhibitory effect of cyproheptadine was expressed as a percentage of the activity of the positive controls in each experiment.

(2) In the second series of experiments mice were injected intraperitoneally with doses between 1 and 100 mg/kg α-methyltyrosine. 2 Hr later, the control group received 0·2 ml water orally, the two other groups 2 mg/kg D-amphetamine and 4 mg/kg D-*p*-chloroamphetamine respectively, in a volume of 10 ml/kg. The activity was again measured 30 min, 1 hr and 2 hr after the application. The gathered activity over the three periods was corrected for the activity of the group that only had received α-methyltyrosine and related to the average activity of the positive controls in series 1.

(3) In the last series of experiments the mice received *p*-chlorophenylalanine in a dose of 300 mg/kg i.p. either 24 hr or both 48 and 24 hr before the experiment. In the mouse this compound displays its maximal depletory effect on the central 5-HT by 24 hr and not after 48 hr as Jéquier *et al.*[16] found in rats. Injection in acid solution (pH about 1·6) proved to be toxic, therefore the compound was injected as a suspension in a vehicle consisting of 8·16 g carboxymethylcellulose 7LF, 1·02 g methyl-*p*-hydroxybenzoate and 8·16 g NaCl/l. distilled water. In this form treatment with *p*-chlorophenylalanine was well tolerated. 24 Hr after the last dose of *p*-chlorophenylalanine the mice were allowed 3–4 courses in the activity cage in order to get a conveniently low basic activity. The control group then received 0·2 ml water by mouth,

the two other groups either D-amphetamine or D-*p*-chloroamphetamine in doses of 2–4 mg/kg orally. The further procedure was as described under 2.

(b) *Influence of the treatment on body temperature.* In some experiments the esophageal temperature of the mice was taken by means of an electric universal thermometer type TE 3 from Ellab A/S, Copenhagen, This was done before as well as 30 min, 1 hr and 2 hr after oral administration of amphetamine and *p*-chloroamphetamine in the doses described

(c) *Determination of central monoamines.* (1) For the determination of noradrenaline and dopamine the brains of two mice were homogenized and extracted as described by Shore and Olin[17] but with the modifications mentioned in our previous paper.[1] The extract was divided and noradrenaline was determined by the modified method of Shore and Olin[1, 17] in the one portion, and dopamine was determined by the method of Fleming *et al.*[18] in the other one. The results were corrected for 80 per cent recovery in the case of noradrenaline and 70 per cent in the case of dopamine.

(2) 5-HT was determined in single brains of mice according to Snyder *et al.*[19] with minor variations.

The spectrophotometry was done with the Zeiss spectrofluorometer with two monochromators (ZMF 4C).

(d) *Drugs used.* D-Amphetamine was used as the sulfate, D-*p*-chloroamphetamine partly as the hydrochloride, partly as the hydrogentartrate. The doses of both amphetamines are given in terms of the base. Cyproheptadine.HCl was a gift from A/S Alfred Benzon, Copenhagen. α-Methyl-*p*-tyrosine and *p*-chlorophenylalanine were synthetized in our Chemical Department by magister S. Rachlin and Dr. P. W. Feit, respectively.

RESULTS

(1) *Central stimulant activity of* D-*amphetamine and* D-p-*chloroamphetamine.* In the previous study,[1] we had estimated the effect of *p*-chloroamphetamine on the locomotor activity of mice to be about half as strong as that of amphetamine. In the present experiments, a dose of 2 mg/kg D-amphetamine gave an average count of 950 (530–1700) (geometrical means ± one S.D. of 8 experiments). In 19 corresponding experiments with 4 mg/kg D-*p*-chloroamphetamine the average number of counts was 600 (350–1000). According to these results, *p*-chloroamphetamine seems only to have about one third of the central stimulant activity of amphetamine.

In the experiments with α-methyltyrosine and *p*-chlorophenylalanine the above mentioned average counts were taken as "100 per cent activities" and the activities after the pretreatment were related to these values.

(2) *Influence of* D-*amphetamine and* D-p-*chloroamphetamine on body temperature.* The effect of the two drugs in the doses used throughout the study on body temperature of mice is summarized in Table 1. It can be seen that *p*-chloroamphetamine was virtually without effect on body temperature whereas amphetamine caused a fall of about 1° at 30 min and 1 hr.

(3) *Influence of cyproheptadine on the locomotor effect of* D-*amphetamine and* D-p-*chloroamphetamine.* Fig. 1. shows that an i.p. dose of 0·11 mg/kg cyproheptadine.HCl inhibited the central stimulant effect of *p*-chloroamphetamine by 50 per cent and was without influence on the effect of amphetamine. Doses higher than 0·125 mg/kg cyproheptadine.HCl did not result in a further depression of *p*-chloroamphetamine

induced activity. The effect of amphetamine could be reduced to 50 per cent by a dose of 0·54 mg/kg cyproheptadine.HCl.

All mice which received 1 mg/kg cyproheptadine.HCl with amphetamine showed piloerection, a fine tremor and small jerking jumps. These jumps were, so to speak, standing jumps and did not involve progression. Mice receiving amphetamine together with lower doses of cyproheptadine did not show these symptoms. On the

TABLE 1. INFLUENCE OF ORAL DOSES OF 2-MG/KG D-AMPHETAMINE AND 4 MG/KG D-*p*-CHLOROAMPHETAMINE ON THE ESOPHAGEAL TEMPERATURE IN MICE

Groups of 6 mice were used. The table gives the mean value ± S.D. in °C.

Treatment	Body temperature				
	Before	30 min after oral application	1 hr after oral application		2 hr
Controls (0·2 ml water)	38·6 ± 0·14	38·6 ± 0·31	38·0 ± 0·63	37·8 ± 0·47	37·2 ± 0·48
Amphetamine	38·7 ± 0·12	38·1 ± 0·41	37·0 ± 0·61	36·6 ± 0·92	37·0 ± 0·77
p-Chloro-amphetamine	38·5 ± 0·43	38·1 ± 0·16	37·7 ± 1·2	37·6 ± 0·96	37·5 ± 0·68

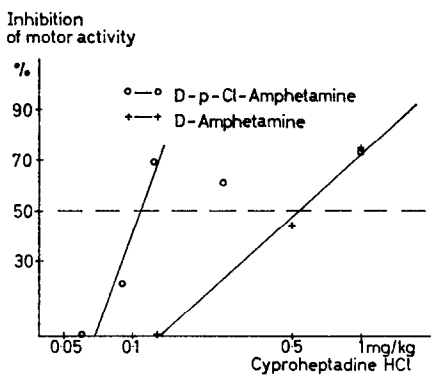

FIG. 1. Inhibitory effect of cyproheptadine on the motor activity evoked by amphetamine and *p*-chloroamphetamine in mice.

other hand, only some of the mice receiving combinations of 0·5 or 1 mg/kg cyproheptadine.HCl with *p*-chloroamphetamine showed these effects.

(4) *Effect of pretreatment with α-methyltyrosine on the locomotor activity produced by* D-*amphetamine and* D-p-*chloroamphetamine.* After the i.p. injection of α-methyltyrosine the mice showed lower exploratory activity than the controls did when they were put in the activity box for the first time. Although this depressant effect of α-methyltyrosine became pronounced only when a dose of 30 mg/kg or larger was used there was nevertheless, an effect even when a dose of only 1 mg/kg was used.

Table 2 shows the brain concentrations of noradrenaline and dopamine determined 2 and 4 hr after the i.p. injection of α-methyltyrosine in doses of 2·5–100 mg/kg. Significant depletions of both these amines occurred with doses down to 5 mg/kg after 2 hr and of 10–20 mg/kg after 4 hr. A dose of 100 mg/kg had no effect on the central 5-HT levels.

TABLE 2. CONCENTRATIONS OF NORADRENALINE AND DOPAMINE IN THE BRAIN OF MICE 2 AND 4 HR AFTER TREATMENT WITH DIFFERENT DOSES OF α-METHYLTYROSINE

The table gives mean value ± S.D., the figures in parentheses are the number of determinations.

α-Methyl-tyrosine mg/kg	2 hr		4 hr	
	Noradrenaline	Dopamine	Noradrenaline	Dopamine
		μg/g		
100	0·19 ± 0·03 (6) P < 0·001	0·31 ± 0·06 (6) P < 0·001	0·25 ± 0·06 (5) P < 0·001	0·24 ± 0·04 (5) P < 0·001
30	0·19 ± 0·03 (5) P < 0·001	0·27 ± 0·09 (5) P < 0·001	0·27 ± 0·06 (4) P < 0·01	0·4 ± 0·13 (4) P < 0·001
20	0·27 ± 0·04 (5) P < 0·01	0·35 ± 0·13 (5) P < 0·001	0·19 ± 0·01 (6) P < 0·001	0·48 ± 0·07 (6) P < 0·01
10	0·23 ± 0·01 (6) P < 0·001	0·55 ± 0·06 (6) P > 0·05	0·27 ± 0·02 (6) P < 0·001	0·59 ± 0·08 (6) P > 0·05
5	0·31 ± 0·01 (6) P < 0·05	0·46 ± 0·07 (6) P < 0·01	0·32 ± 0·01 (6) P > 0·05	0·57 ± 0·02 (6) P > 0·05
2·5	0·33 ± 0·02 (6) P > 0·05	0·55 ± 0·02 (6) P > 0·05	0·35 ± 0·04 (6) P > 0·05	0·7 ± 0·11 (6) P > 0·05
Controls	Noradrenaline: 0·35 ± 0·04 μg/g (18)			
	Dopamine: 0·65 ± 0·13 μg/g (18)			

Fig. 2 shows the reduction of locomotor activity provoked by the two amphetamines in relation to the dose of α-methyltyrosine used. A reduction of the stimulant effect of amphetamine by 50 per cent is achieved with a dose of 4·2 mg/kg, and even with 2·5 mg/kg some reduction in motor activity is apparent though this dose is

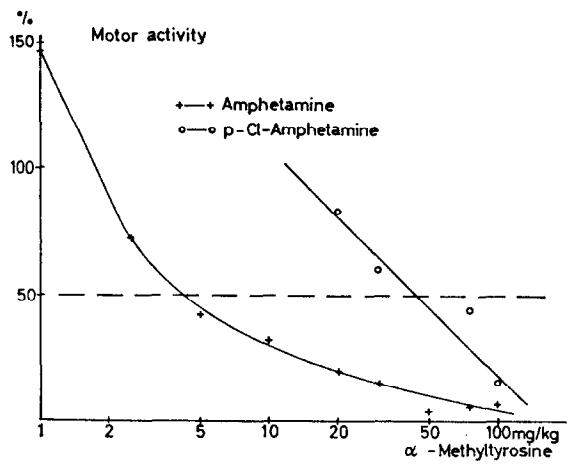

FIG. 2. Effect of a pretreatment with α-methyltyrosine on the motor activity evoked by amphetamine and *p*-chloroamphetamine in mice.

practically without effect on the central concentration of the catecholamines. In order to depress the locomotor activity after *p*-chloroamphetamine by 50 per cent, a dose of 44 mg/kg was necessary. Doses of this order of magnitude lead to pronounced depletions of the central catecholamines.

(5) *Influence of pretreatment with* p-*chlorophenylalanine on locomotor activity produced by* D-*amphetamine and* D-p-*chloroamphetamine.* Mice that were pretreated once or twice with *p*-chlorophenylalanine made a very alert and active impression. Although their initial exploratory activity was no higher than that of the untreated controls it declined more slowly than it did in these.

The influence of treatment with 300 mg/kg *p*-chlorophenylalanine 24 hr or 48 and 24 hr (i.e. once or twice) before the experiment on the central 5-HT levels in mice is shown in Table 3. Under these conditions the compound had no influence on central catecholamine concentrations. Treatment with the amphetamines did not depress the central 5-HT concentrations further than *p*-chlorophenylalanine alone.

24 Hr after a single dose of 300 mg/kg *p*-chlorophenylalanine the locomotor activity produced by amphetamine was practically unaltered while that after *p*-chloroamphetamine was a little enhanced (Table 4). After pretreatment with this dose on

TABLE 3. CONCENTRATIONS OF 5-HT IN THE BRAIN OF MICE AFTER PRETREATMENT WITH *p*-CHLOROPHENYLALANINE, 300 MG/KG ONCE OR TWICE 24 HR OR 48 AND 24 HR BEFORE THE EXPERIMENT

The table gives mean value ± S.D. Number of determinations in parentheses.

Treatment	5-HT in μg/g	P	
Controls	0·48 ± 0·04 (8)		
p-Chlorophenylalanine			
1×	0·26 ± 0·06 (18)	<0·001	<0·01
2×	0·20 ± 0·04 (18)	<0·001	

TABLE 4. INFLUENCE OF PRETREATMENT WITH *p*-CHLOROPHENYLALANINE ON THE MOTOR ACTIVITY EVOKED BY D-AMPHETAMINE AND D-*p*-CHLOROAMPHETAMINE IN MICE

The results are given in percent of the locomotor activity after, respectively, 2 mg/kg D-amphetamine and 4-mg/kg D-*p*-chloroamphetamine in otherwise untreated mice (geometrical means and range for one S.D.). Pretreatment was with 300 mg/kg *p*-chlorophenylalanine once, 24 hr, or twice, 48 and 24 hr before the experiment.

Treatment	Motor activity after	
	Amphetamine	*p*-Chloroamphetamine
once	90 (48–170)	140 (97–200)
twice	130 (81–210)	13*

* In 2 experiments the activity lay below that of the control group treated only with *p*-chlorophenylalanine, in the third experiment 56 per cent of the activity of mice treated only with *p*-chloroamphetamine were reached.

two preceding days the activity after amphetamine seemed somewhat enhanced. The effect of *p*-chloroamphetamine on the other hand was nearly abolished

DISCUSSION

The results described support the idea that amphetamine and its *p*-chloro analogue depend on different mediators of their central action though the differences in the pharmacological properties displayed by both drugs are of a quantitative nature only.

Cyproheptadine was chosen as the compound for blocking 5-HT receptors since this drug exerts its 5-HT blocking effect in a dose range lying clearly below the adrenolytic one.[20] The inhibitory effect of the drug on the central stimulation produced by *p*-chloroamphetamine was dose-dependent in the range of 0·06–0·125 mg/kg. Such dose levels must be considered to be below those producing measurable adrenolytic activity. A considerable antagonism to the effect of amphetamine was first apparent with doses of 0·5 and 1 mg/kg cyproheptadine which lie in the adrenolytic dose range.[20] The objection that cyproheptadine might have a depressant effect of its own, thus antagonizing the weak stimulant effect of *p*-chloroamphetamine at lower doses than those counteracting the stronger effect of amphetamine seems to be irrelevant as pretreatment with doses up to 0·5 mg/kg cyproheptadine has been shown to be without influence on the hexobarbital sleeping time in mice. A dose of 1 mg/kg led in only one out of two experiments to a just significant prolongation of the sleeping time.

The inhibition of the locomotor effect of amphetamine by pretreatment with α-methyltyrosine in our experiments is in excellent agreement with the previous results of Weissman *et al.*[7] In both studies, a significant inhibition was already apparent at doses of 2·5 and 3 mg/kg, respectively. These doses have only a very weak effect on central catecholamines after 2 hr, and are without effect after 4 hr (Table 2). Since the amphetamine effect is thus inhibited in spite of near normal amounts of noradrenaline being available, amphetamine might be dependent on freshly synthesized noradrenaline (the "functional pool" of Weissman *et al.*[7]) in order to display its central stimulant effect. This assumption could explain the central stimulant effect of amphetamine in animals whose noradrenaline stores have been severely depleted by reserpine since this drug only has a limited effect on the biosynthesis of the adrenergic transmitter in brain,[21] and freshly synthesized noradrenaline should thus be available.

The effect of *p*-chloroamphetamine was first inhibited by doses of α-methyltyrosine 10 times higher than those inhibiting amphetamine, and which provoke a pronounced depletion of the central catecholamines.

p-Chlorophenylalanine has been shown to be a specific inhibitor of tryptophan hydroxylase, thus depleting the 5-HT stores.[16,22] The effect is long lasting since the inhibition is irreversible *in vivo*.[16] Welch and Welch[23] have shown that the compound may also have an influence on central noradrenaline levels in some strains of mice, but this was not the case in the strain used by us when measured 24 hr after the last injection. One injection of 300 mg/kg *p*-chlorophenylalanine lowered the central 5-HT by 46 per cent but had no effect on the central stimulant activity of the two amphetamines. However, after two injections of the inhibitor, 5-HT depletion amounted to nearly 60 per cent, and the central stimulant effect of *p*-chloroamphetamine was totally abolished in two out of three experiments and was considerably reduced in the third. On the other hand, the activity of amphetamine seemed even to be enhanced.

The results permit a conception on the mode of action of both compounds which is outlined in Fig. 3.

The locomotor effect of amphetamine can be inhibited by small doses of α-methyltyrosine as well as by relatively high (adrenolytic) doses of cyproheptadine but not by *p*-chlorophenylalanine. Since the doses of α-methyltyrosine which inhibit the effect of amphetamine have virtually no effect in depleting central stores of noradrenaline, but nevertheless probably reduced the amount of freshly synthesized noradrenaline for a brief period, it was concluded that amphetamine must be dependent

on this freshly synthesized catecholamine (NA 1 in Fig. 3) in order to display its central stimulant effect. It has already been mentioned that this conception may explain the central effect of amphetamine after reserpine without postulating a direct action on the adrenergic receptor. The possible role of dopamine as a mediator of central amphetamine effects is neglected in this connection since it is felt that the role of dopamine is primarily confined to the gnawing compulsion and the stereotypic reactions caused by higher doses of amphetamine.[12, 24]

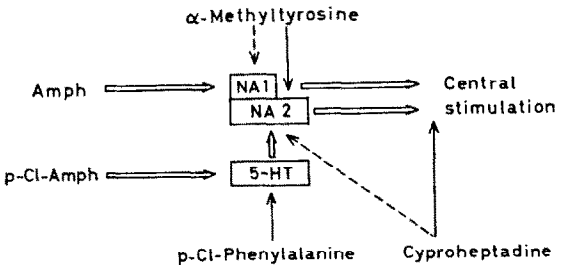

FIG. 3. Assumed mode of action of the central stimulant effect of amphetamine and *p*-chloroamphetamine. Small doses of the inhibitors are characterized by interrupted, higher doses by uninterrupted arrows. For further explanation see text.

p-Chloroamphetamine seems to be dependent both on 5-HT and noradrenaline (or dopamine?) as mediators of its central stimulant effect since the latter can be abolished by small doses of the 5-HT antagonist cyproheptadine, or by a severe depletion of central 5-HT as well as by a pronounced depletion of catecholamines produced by higher doses of α-methyltyrosine. These facts fit the diagram of Fig. 3 according to which *p*-chloroamphetamine first releases 5-HT which in turn liberates noradrenaline (or another catecholamine?) from a store (NA 2) that is not identical with freshly synthesized noradrenaline (NA 1) which is considered to be important for amphetamine. The noradrenaline liberated by 5-HT should then be the final mediator also of the central stimulant effect of *p*-chloroamphetamine.

An explanation of the different modes of action of two compounds so closely related as amphetamine and its *p*-chloro analogue seems difficult. However, it has been shown that the central concentrations of *p*-chloroamphetamine greatly exceed those after an equal dose of amphetamine and are maintained for a longer time.[1] This is probably both the consequence of the more lipophilic character of the *p*-chloro compound and its stronger binding to the particulate fraction in the CNS.[25] Furthermore, the *p*-chlorophenyl moiety seems to have a special affinity to sites of biosynthesis and storage of 5-HT: e.g. *p*-chlorophenylalanine is an inhibitor of tryptophan hydroxylase, and the *p*-chloroamphetamines deplete central 5-HT stores.[2, 3] It is of interest in this connection that the mouse is the only species in which a depletion of central 5-HT by *p*-chloromethamphetamine could not be shown.[2] The results of the present investigation show, however, that this species difference cannot be regarded as a fundamental one, and possibly is caused by a more rapid biosynthesis of the amine in the mouse. Last but not least, the inhibitory effect of *p*-chloroamphetamine on monoamine oxidase must be considered. This effect is quantitatively of the same order of magnitude as that of amphetamine,[1, 26] but, considering the much higher concentrations and a different subcellular distribution[25] of the *p*-chloro compound in the CNS,

it may play some part in the biochemical mode of action, for example by raising the concentration of free 5-HT at the site where we assume this amine liberates noradrenaline.

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