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# EFFECT OF AMMONIUM CHLORIDE ON THE POTENTIATION OF AMPHETAMINE BY PSYCHOTROPIC DRUGS IN THE RAT

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**Abstract**—The effect of acidification of the urine on the potentiation of amphetamine by imipramine, butriptyline and chlorpromazine was investigated in the rat. Urine was acidified by administering ammonium chloride (500 mg/kg p.o.). Amphetamine, imipramine, butriptyline and chlorpromazine were injected i.p. CNS stimulation was measured in jiggle cages. Amphetamine and *p*-hydroxyamphetamine excretion in the urine were determined 4 hr after administration of the drugs. Ammonium chloride increased the rate of urinary excretion of amphetamine and shortened the intensity and the duration of amphetamine hypermotility. These findings correlated with lower levels of brain amphetamine.

In rats treated with the combinations of amphetamine with (1) imipramine, (2) butriptyline and (3) chlorpromazine, ammonium chloride abolished the potentiation and prolongation of amphetamine effects. The effect of ammonium chloride on amphetamine excretion and brain levels in these groups was similar to that observed in animals treated with amphetamine alone. It is suggested that ammonium chloride blocked the potentiation of amphetamine stimulation by the psychotropic agents by promoting a more rapid excretion of amphetamine.

POTENTIATION of the central effects of amphetamine by tricyclic antidepressants and chlorpromazine has been reported by several authors[1–4] and the mechanism of the potentiation has been also extensively investigated. Sulser *et al.*[5] have shown that desimipramine inhibited the formation of *p*-hydroxyamphetamine, which is the major metabolite of amphetamine in the rat.[6] Their data were confirmed and a similar mechanism was reported for chlorpromazine.[7–9] As a consequence of the inhibition of metabolism, the antidepressants and chlorpromazine increased the level of amphetamine in all the tissues tested: brain, serum and liver.[7–9] On the other hand, Asatoor *et al.*[10] have shown that the excretion of unmetabolized amphetamine in the rat was significantly increased by acidification of the urine with ammonium chloride and that the excretion of *p*-hydroxyamphetamine was not influenced by the pH of the urine.

In the present work, the effect of ammonium chloride on the interaction between tricyclic antidepressants and amphetamine and also chlorpromazine and amphetamine was studied. Two tricyclic antidepressants were used: imipramine[11] and butriptyline.[12]

## MATERIALS AND METHODS

Male albino rats (150 ± 10 g) of the Charles River strain were used in all experiments. The animals were fasted for 18 hr before the experiments, but received water *ad lib.* General activity was measured in cylindrical jiggle cages.[13] The rats were placed in individual cages at 9.00 a.m. and left there for 1 hr for acclimatization without recording their activity. After this period, the drugs were administered and the

rats were returned to their cages. Recording of motility was started 45 min after drug administrations and continued for 6 hr at hourly intervals using 15 rats per treatment group. In some experiments motility was recorded for 12 hr. The experiments were conducted in a temperature controlled room under artificial lighting.

Brain levels of amphetamine were determined by a modification of the colorimetric method of Axelrod,[6] 4 hr after administration of the drugs. The modification was introduced to avoid interference by chlorpromazine. This was done by adding perchloric acid (to obtain a concentration of 6%) to the brain homogenate before the first benzene extraction.[9] The same procedure was used for the determination of amphetamine in the urine. Urinary total *p*-hydroxyamphetamine (free plus esterified) was determined by the method of Axelrod.[6] Urine samples were collected during 4 hr after drug administration.

The following compounds were used: *dl*-amphetamine sulfate (May & Baker Ltd.), imipramine HCl (Geigy), chlorpromazine HCl (Polenc), ammonium chloride and ammonium carbonate (Fisher), butriptyline HCl.[12] All the psychotropic agents were injected in physiological saline. All doses are expressed as the salts. Ammonium chloride and ammonium carbonate were administered p.o. in standard doses of 500 mg/kg immediately before the i.p. injections of the other drugs. The doses of ammonium salts were administered in 1·5 ml water. Similar amounts of water (10 ml/kg) were administered to all the other groups.

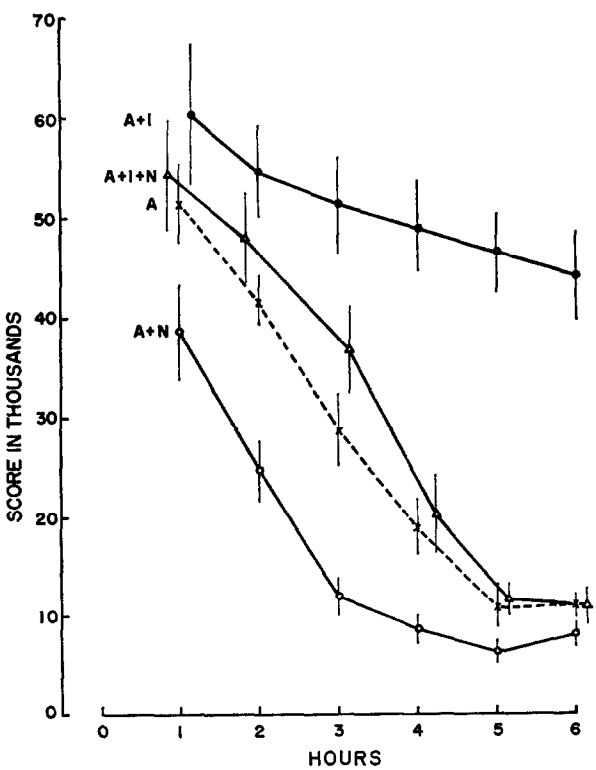


FIG. 1. Effect of ammonium chloride (N), 500 mg/kg p.o., on the hypermotility induced by amphetamine (A), 5·8 mg/kg i.p., alone and in combination with imipramine (I), 5 mg/kg i.p.

## RESULTS

The effect of ammonium chloride administration on the hypermotility produced by amphetamine and by the combination of imipramine and amphetamine is shown in Fig. 1. The hourly motility scores of water-treated rats (control group), which are not shown on the figure, ranged from 8000 to 15,000 counts per hr.

The hypermotility caused by 5·8 mg/kg of amphetamine was greatest during the first hour and by the fifth hour the motility of this group was in the range of the control rats. Imipramine (5 mg/kg) potentiated both the intensity and the duration of amphetamine stimulation. The motility of rats treated with this combination returned to control levels in 10–12 hr. Ammonium chloride treatment antagonized both the intensity and the duration of amphetamine stimulation. Furthermore, stimulation caused by the combination of amphetamine and imipramine was considerably reduced by ammonium chloride and the hourly motility scores of this treatment group were not significantly different from those of the amphetamine group.

Similar results were obtained with the combination of 5 mg/kg of butriptyline and amphetamine (Fig. 2).

Figure 3 shows the effect of ammonium chloride on the motility by amphetamine and by the combination of amphetamine and chlorpromazine. In these experiments, the sensitivity of the motility recorders was about 30 per cent less than in the previous

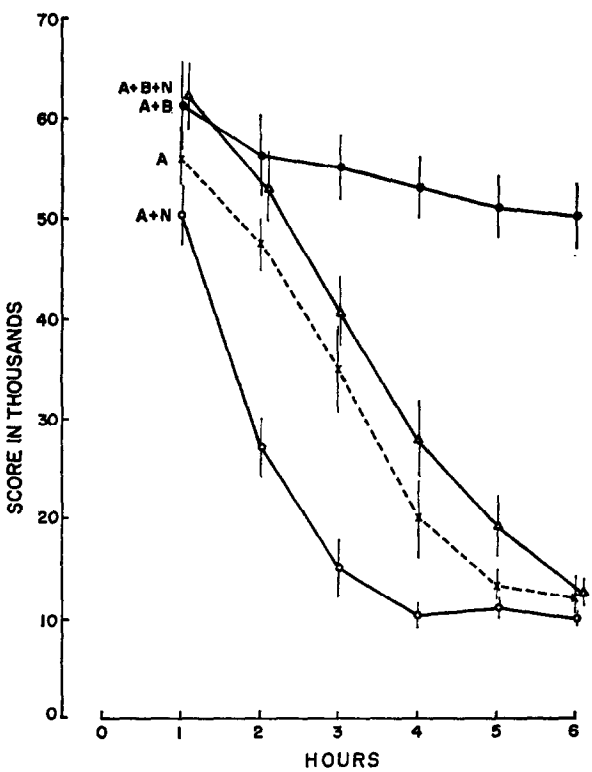


FIG. 2. Effect of ammonium chloride (N), 500 mg/kg p.o., on the hypermotility induced by amphetamine (A), 5·8 mg/kg i.p., alone and in combination with butriptyline (B), 5 mg/kg i.p.

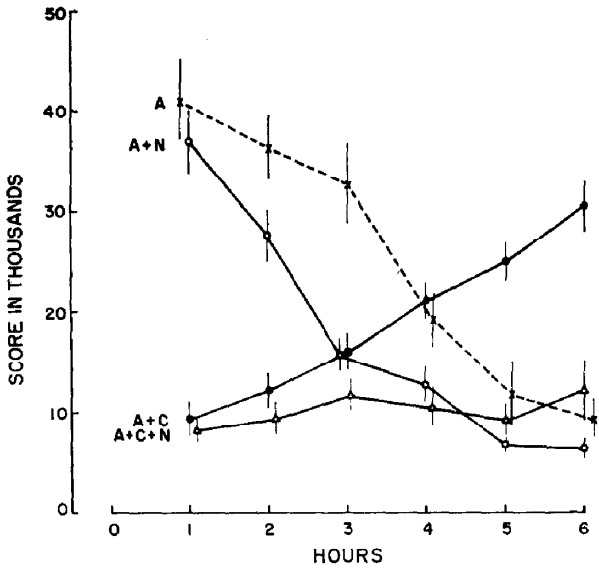


FIG. 3. Effect of ammonium chloride (N), 500 mg/kg p.o., on the hypermotility induced by amphetamine (A), 5·8 mg/kg i.p., alone and in combination with chlorpromazine (C), 4 mg/kg i.p.

measurements. The motility scores for saline-treated controls ranged between 5000 and 10,000 per hr in this series. Chlorpromazine (4 mg/kg) antagonized amphetamine hypermotility initially and this combination caused a delayed stimulation, as reported earlier.[9] When ammonium chloride was added to the combination of amphetamine and chlorpromazine, there was a complete antagonism of the amphetamine stimulation. The motility scores of rats which received these three drugs did not differ from the scores of controls up to 12 hr after administration.

The effect of ammonium chloride on the brain levels of amphetamine in rats submitted to different treatments is shown in Table 1. Four hr after the administration of 11·6 mg/kg of amphetamine, there was 2·06 $\mu$g amphetamine per g of brain. All three psychotropic agents caused significant increases in the brain levels of amphetamine. In the doses tested, imipramine resulted in the highest brain level (11·6 $\mu$g/g) followed by butriptyline (9·1 $\mu$g/g).

The addition of ammonium chloride to all treatment groups resulted in significant decreases in the levels of brain amphetamine. With the exception of the combinations with chlorpromazine, there was a good correlation between the brain levels of amphetamine and the psychomotor stimulation as shown in Figs. 1, 2 and 3. In the chlorpromazine combination group, there was a high brain level of amphetamine but no stimulation.

The urinary excretion of amphetamine, expressed in micrograms per 100 g of body weight, is shown in Table 1 (column 3). Four hr after the administration of amphetamine, there was 203 $\mu$g of unchanged amphetamine excreted in the urine. Chlorpromazine and butriptyline caused significant increases ($P < 0{\cdot}001$) in the excretion of amphetamine, while the increases caused by imipramine were statistically not significant. Administration of ammonium chloride caused significant ($P < 0{\cdot}001$) increases in the urinary excretion of amphetamine in all treatment groups.

TABLE 1. EFFECT OF AMMONIUM CHLORIDE (500 mg/kg p.o.) ON BRAIN LEVELS AND URINARY EXCRETION OF AMPHETAMINE IN RATS 4 hr AFTER i.p. ADMINISTRATION OF AMPHETAMINE (11·6 mg/kg) ALONE AND IN COMBINATION WITH CHLORPROMAZINE (8 mg/kg), IMIPRAMINE (5 mg/kg) AND BUTRIPTYLINE (5 mg/kg)

| Treatment | Brain level of amphetamine (μg/g) | Urinary excretion | | | |
|---|---|---|---|---|---|
| | | Amphetamine (μg/100 g body wt.) | *p*-OH-amphetamine (μg/100 g body wt.) | Urine in 4 hr (ml/100 g) | pH of urine |
| Amphetamine | 2·06 ± 0·13 (8) | 203 ± 12 (9) | 118 ± 8 (5) | 3·1 ± 0·2 (21) | 7·2 ± 0·03 (38) |
| Amphetamine + $NH_4Cl$ | 0·19 ± 0·06 (11) | 417 ± 12 (12) | 110 ± 12 (9) | 3·2 ± 0·2 (12) | 5·9 ± 0·09 (12) |
| Amphetamine + imipramine | 11·6 ± 0·6 (4) | 296 ± 44 (4) | 13·3 ± 0·6 (4) | 3·4 ± 0·3 (12) | 7·2 ± 0·07 (16) |
| Amphetamine + imipramine + $NH_4Cl$ | 1·84 ± 0·16 (6) | 706 ± 23 (9) | 6·4 ± 0·9 (9) | 3·5 ± 0·2 (14) | 6·0 ± 0·08 (12) |
| Amphetamine + butriptyline | 9·1 ± 1·0 (7) | 304 ± 13 (4) | 25·5 ± 1·2 (7) | 3·5 ± 0·2 (14) | 7·3 ± 0·08 (12) |
| Amphetamine + butriptyline + $NH_4Cl$ | 2·3 ± 0·4 (7) | 651 ± 24 (7) | 16·4 ± 2·4 (7) | 3·4 ± 0·2 (11) | 5·9 ± 0·05 (11) |
| Amphetamine + chlorpromazine | 7·38 ± 4·2 (6) | 373 ± 17 (6) | 3·7 ± 0·8 (6) | 2·4 ± 0·1 (19) | 6·6 ± 0·05 (22) |
| Amphetamine + chlorpromazine + $NH_4Cl$ | 3·7 ± 0·3 (5) | 631 ± 34 (6) | 4·7 ± 0·7 (6) | 2·9 ± 0·1 (21) | 5·9 ± 0·04 (21) |

* Results are expressed as the means ± S.E.M. Numbers in parentheses indicate the number of determinations.

Urinary excretion of *p*-hydroxyamphetamine is shown in Table 1 (column 4). After amphetamine treatment, there was 118 μg *p*-hydroxyamphetamine excreted. All three psychotropic agents caused marked decreases in the excretion of *p*-hydroxyamphetamine. In the doses tested, chlorpromazine caused the greatest inhibition followed by imipramine. Ammonium chloride did not affect the excretion of *p*-hydroxyamphetamine in the amphetamine and amphetamine–chlorpromazine group. In the amphetamine–imipramine and in the amphetamine–butriptyline groups, ammonium chloride caused significant reductions in the *p*-hydroxyamphetamine content of the urine samples.

The amounts of urine excreted in 4 hr are shown in Table 1. Control rats receiving 10 ml/kg of water only (not shown in the Table) excreted 2·5 ± 0·1 ml per 100 g, as found in 17 determinations. Amphetamine-treated rats excreted 3·1 ± 0·2 ml urine and additional treatment with ammonium chloride did not increase the output. Ammonium chloride alone had a significant diuretic effect (3·6 ± 0·3 ml/100 g). The

effect of amphetamine on urine output was antagonized by chlorpromazine but not by imipramine and butriptyline. The excretion of amphetamine did not depend on the amount of urine voided.

The pH of the urine of the control rats was 6·4 ± 0·1 in 34 samples. The effects of drug treatments are shown in the last column of Table 1. Rats treated with amphetamine excreted urine of pH 7·2. This effect of amphetamine was antagonized by chlorpromazine and the pH of the urine of rats treated with this combination was in the range of water controls. Rats treated with the combination of amphetamine and imipramine or amphetamine and butriptyline excreted slightly alkaline urines of pH 7·2 and 7·3 respectively. Ammonium chloride acidified the urine of all treatment groups.

In order to ascertain whether ammonium chloride exerted the reported pharmacological and biochemical effects through acidification of the urine, control experiments were performed with ammonium carbonate. A dose of 500 mg/kg of ammonium carbonate p.o. did not change the pH of the urine, but loaded the organism with ammonium ions. This treatment did not block the potentiation of amphetamine stimulation by imipramine and had no appreciable effect on the excretion of amphetamine.

## DISCUSSION

Our results confirm the findings of Asatoor *et al.*[10] that acidification of the urine with ammonium chloride increased the amount of unchanged amphetamine in the urine without affecting the excretion of *p*-hydroxyamphetamine. Furthermore, it was found that ammonium chloride diminished and shortened the CNS stimulation induced by amphetamine and that this effect correlated well with the lower levels of amphetamine in the brain.

In the search for new antidepressants, the potentiation of the central effects of amphetamine in rats is frequently used as a testing method.[14–16] As the result of several investigations,[5,8,9] it is apparent that chlorpromazine and the tricyclic antidepressants potentiate the CNS effects of amphetamine not by a central mechanism but by the hepatic inhibition of its metabolism.[17] Chlorpromazine and the tricyclic antidepressants belong to different pharmacological classes, but their effects on the metabolism of amphetamine are very similar.

In the present investigation, the potentiation of the central effects of amphetamine by the psychotropic agents was antagonized by a peripheral effect, namely increased urinary excretion of the stimulant. The administration of ammonium chloride acidified the urine and accelerated the excretion of unmetabolized amphetamine. The excretion of amphetamine was particularly high in rats which received both ammonium chloride and a psychotropic agent. This was probably due to the higher amounts of amphetamine present in the organism as a result of the inhibition of metabolism of amphetamine by the psychotropic agents.

Atropine and scopolamine also potentiate psychomotor stimulation by amphetamine,[18,19] but they do not increase the brain level of amphetamine.[20] It appears that there are at least two mechanisms by which amphetamine effects in the CNS are potentiated. One is due to an increased level of amphetamine in the brain, the other might be the result of a sensitization of the CNS to amphetamine. It is interesting that

chlorpromazine and the antidepressants, which are obviously CNS agents, potentiate amphetamine by increasing its concentration in the brain. On the other hand, atropine, which has no therapeutic application as a CNS agent, seems to sensitize to amphetamine.

In the combination of amphetamine with psychotropic agents and ammonium chloride, the degree of hypermotility correlated well with the brain levels of amphetamine. In the combination of amphetamine with chlorpromazine, there was a high level of brain amphetamine but no hypermotility. This can be explained by the antagonism of amphetamine effect by large doses of chlorpromazine. Small doses of chlorpromazine potentiate amphetamine stimulation and increase the brain level of amphetamine.[9,21]

Asatoor *et al.*[10] reported that ammonium chloride did not affect the excretion of *p*-hydroxyamphetamine. In the present work, this was the case for amphetamine and the chlorpromazine–amphetamine combination. In the combination of amphetamine with the antidepressants, ammonium chloride caused significant decreases of *p*-hydroxyamphetamine excretion. These decreases may have resulted from the lower amounts of amphetamine available to the inhibited hydroxylases due to excretion of very large amounts of unchanged amphetamine. However, it remains unexplained why the chlorpromazine–amphetamine group did not show this effect.

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