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AMMONIA DETOXICATION BY PYRROLIDONECARBOXILATE-ARGININE MIXTURE

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Abstract—The protective effect of pyrrolidonecarboxilate, arginine and their mixture against acute ammonia intoxication by an LD_{50} of ammonium acetate has been investigated in rats. Evaluation of the efficacy of the different treatments has been done on the basis of mortality as well as of blood ammonia and urea levels. Pyrrolidonecarboxilate-arginine mixture was the most effective treatment in acute ammonia intoxication: possible mechanism of action is formulated.

ARGININE has been found to be a very efficient compound in ammonia detoxication.[1, 2] The mechanism by which arginine displays this effect seems to be related to an enhancement of Krebs–Henseleit cycle[3] by a greater conversion of toxic ammonia into urea.[4]

The enzymatic formation of pyrrolidonecarboxilate from glutamate has been demonstrated.[5, 6]

Furthermore, since PC is a side product in the synthesis of glutamine from glutamate and ammonia,[5, 7] it might be possible that high concentrations of PC reverse the reaction IIb

$$\text{(I) ATP} + \text{glutamic acid} + \text{enzyme} \rightleftharpoons \text{ATP-glutamate-enzyme}$$

$$\text{(IIa) ATP-glutamate-enzyme} \underset{-NH_3}{\overset{+NH_3}{\rightleftharpoons}} \text{glutamine} + \text{ADP} + \text{Pi} + \text{enz.}$$

$$\text{(IIb) ATP-glutamate-enzyme} \rightharpoonup \text{PC} + \text{ADP} + \text{Pi} + \text{enz.}$$

giving rice to a glutamate-enzyme, that in turn (by the reaction IIa) traps ammonia by synthesis of glutamine.

The present paper reports studies of the effect of PC in acute ammonia intoxication, as well as its effect when administered in addition to arginine. Such effect has been investigated on the basis of mortality of rats and of their ammonia and urea blood levels.

EXPERIMENTAL

Chemicals. L-arginine was obtained from British Drug Houses Ltd., or from Sigma Chemical Co., L-5-pyrrolidon-2-carboxilic acid was purchased from K & K Laboratories, Inc. Purified urease was Type II from Sigma Chemical Co. All the other chemicals were analytical-grade preparations obtained from usual commercial sources.

The following abbreviation is used: PC ≑ L-pyrrolidonecarboxilic acid.

Nessler's reagent was prepared following Vanselow's directions.[8] Saturate borate-NaOH buffer for determination of ammonia by microdiffusion was prepared according to Reinhold and Chung.[9] Glass distilled water was passed through ion exchange resin as Amberlite IR 120 (H^+ form) or through acid-treated permutite in order to avoid the presence of ammonia traces.

Animals. About 650 female albino rats (Wistar strain) weighing 80–100 g were used. The animals were fasted for 14–16 hr with water *ad libitum*, prior to study.

Treatment. Two i.p. injections (see Tables 1 and 2), at 1 hr interval, were given to each rat. The volume of injected solutions (adjusted to neutrality, when necessary,

TABLE 1. EFFECT OF ARGININE, PYRROLIDONECARBOXILATE AND PYRROLIDONECARBOXILATE-ARGININE MIXTURE IN ACUTE AMMONIA INTOXICATION

Group	Treatment*	No. of rats	Survivors	Mortality (%)	P value
A	Saline + AmAc	150	75	50	
B	Arg + AmAc	120	80	33	<0·01 (vs. A)
C	PC + AmAc	110	57	48	
D	PC-Arg + AmAc	130	108	17	<0·01 (vs. B)

* Saline: NaCl 0·9%; AmAc: ammonium acetate 8·6 m-mole/kg; Arg: L-arginine 0·5 m-mole/kg; PC: L-pyrrolidonecarboxilate 0·5 m-mole/kg; PC-Arg: L-pyrrolidonecarboxilate-L-arginine 0·5 m-mole/ kg each.

TABLE 2. BLOOD AMMONIA AND UREA LEVELS IN RATS INTOXICATED WITH AMMONIUM ACETATE AND PREVIOUSLY PROTECTED WITH ARGININE, OR PYRROLIDONECARBOXILATE OR PYRROLIDONECARBOXILATE-ARGINE MIXTURE

Group	No. of animals	Treatment*	Blood ammonia (A) μmole/ml ± e.s.	P value for A	Blood urea (B) μmole/ml ± e.s.	P value for B
1	19	Saline + Saline	0·39 ± 0·03		5·02 ± 0·39	
2	17	PC + Saline	0·40 ± 0·04		5·38 ± 0·71	
3	20	Arg + Saline	0·44 ± 0·04		5·97 ± 0·58	>0·05 (vs. 1)
4	15	PC-Arg + Saline	0·47 ± 0·04		5·80 ± 0·41	
5	18	Saline + AmAc	5·90 ± 0·23		6·26 ± 0·24	
6	19	PC + AmAc	4·44 ± 0·16	<0·001 (vs. 5)	6·22 ± 0·42	
7	20	Arg + AmAc	3·11 ± 0·22	<0·001 (vs. 5)	7·61 ± 0·32	<0·001 (vs. 5)
8	18	PC-Arg + AmAc	1·56 ± 0·24	<0·001 (vs. 7)	9·08 ± 0·28	<0·001 (vs. 7)

* See Table 1.

with KOH) was equal to 10 ml/kg body wt. The animals used in the experiments summarized in Table 2 were killed by decapitation 15 min after the last injection. The blood from the animals was collected in heparinized test-tubes, kept at 0° until the determination of ammonia and urea (performed however within 20 min following the blood collection).

Methods. Ammonia was determined on aliquots of blood specimens by the micro-diffusion technique[10] according to Cedrangolo *et al.*[11] On separate aliquots of blood specimens urea was determined by urease method. The enzymatic hydrolysis of urea was obtained by addition to each sample of 250 μg of purified urease dissolved in

0·5 ml of 0·2 M acetate buffer, pH 5·6 and taking the samples at 37° for 30 min. In preliminary experiments it has been found that 250 μg of urease were sufficient to hydrolize totally about 3 μmole urea in the indicated experimental conditions.

Statistical methods. The arithmetical mean and S.E.M. were calculated for each group of experiments. Furthermore, χ^2 and Student's t tests were used to assess the significance of difference between the results reported in Tables.[12]

RESULTS

Table 1 shows the results of the experiments carried out on 4 groups of rats. Each group of animals was observed for mortality within 6 hr from the last injection. It is possible to see that arginine, in dose of 0·5 m-mole/kg, reduced mortality of intoxicated rats from 50 to 33 per cent (χ^2 test between groups A and B was equal to 6·71; $P < 0·01$).

No changes in per cent of mortality were observed when PC alone was given to rats in a dose of 0·5 m-mole/kg (see groups A and C).

The injection of PC-arginine mixture in dose of 0·5 m-mole/kg each provoked a more definite protective effect: mortality was lowered from 50 to 17 per cent. The decrease of mortality observed with the administration of PC-arginine mixture was clearly significant when compared to protective effect exhibited by arginine alone (χ^2 test between groups B and D was equal to 7·74; $P < 0·01$).

In order to explain the described results, the experiments summarized in Table 2 have been performed. PC or arginine alone and PC-arginine mixture (groups 2–4) administered to normal rats did not change either blood ammonia or urea levels. In intoxicated animals, the treatment with arginine produced a decrease of blood ammonia levels and higher blood urea concentration. The differences between ammonia and urea blood levels of intoxicated animals (group 5) and those of rats previously treated with arginine (group 7) exhibited P values $<0·001$. PC, when administered alone, while provoking no changes in urea levels, decreased significantly ($P < 0·001$) the ammonia levels (group 6 compared to group 5).

The protective effect of PC-arginine mixture against ammonia intoxication was clearly shown by the results obtained in group 8: the ammonia levels fall to about 1/4 when compared to the corresponding values of group 5 ($P < 0·001$). The same level of significance ($P < 0·001$) was observed when ammonia levels of rats of group 8 (PC-arginine mixture) were compared with those of the animals of group 7 (arginine).

At the same time the blood urea levels appeared to be significantly increased ($P < 0·001$) when group 8 was compared to group 5 and also when group 8 was compared to group 7.

DISCUSSION

The protective effect of arginine against acute ammonia intoxication seems to be related, in agreement with the findings of other authors,[4] to an enhancement of urea synthesis mechanism: this is indicated by fall in blood ammonia and parallel increase of blood urea levels.

On the basis of mortality, PC alone shows no protective effect in the used experimental conditions; while on the basis of blood ammonia levels reduction it shows a significant decrease that, however, is not sufficient to reduce the mortality. The fact that no increase in urea levels is parallely observed supports the hypothesis that PC

administered alone is able to detoxicate ammonia by its conversion to a compound that is not urea. This could probably be identified with glutamine as indicated in introduction (see ref. 7).

The administration of PC-arginine mixture, however, provokes a more definite reduction of ammonia levels as well as of mortality of rats together with an increase of urea levels. The reduction of ammonia levels appears to be practically equal to the sum of those provoked by arginine and PC alone.

On the contrary, the increase of urea levels obtained by administration of PC-arginine mixture is higher than that observed with arginine alone (PC alone does not increase urea levels). This greater urea synthesis exhibited by arginine when also PC is present could be explained on the basis of a conversion into urea of both ammonia and glutamine amido nitrogen[13] (see Fig. 1). However, it has to be considered that the direct conversion of glutamine amido-nitrogen into urea has not been jet proved in mammalian organism.

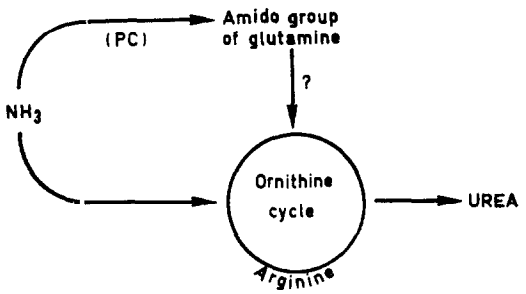

FIG. 1. Tentative mechanism for ammonia detoxication by pyrrolidonecarboxilate-arginine mixture.

Therefore, it may be inferred that the mechanism of ammonia detoxification by PC-arginine mixture might be caused by two different mechanisms; (i) glutamine synthesis from PC and ammonia; (ii) urea synthesis from glutamine (amido group) and ammonia, both enhanced by the presence of arginine. However, other mechanism of ammonia detoxification by PC cannot be excluded.

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