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Acute effects of ethanol and acetaldehyde on plasma phosphate level

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Oral administration of ethanol in a dose of 65 mmol kg^{-1} produced marked change of plasma phosphate level in rabbits. Hypophosphataemia was observed for the first 2 h after administration followed by significant increase of plasma phosphate at 5 h. Hypophosphataemia did not appear when ethanol was given to the rabbits pretreated with pyrazole. When animals were injected with disulfiram in advance, the duration of hyperphosphataemia due to ethanol was prolonged. Administration of acetaldehyde at a dose of 1·5 mmol kg^{-1} produced hyperphosphataemia. In this study, plasma phosphate was not associated with change in calcium level. These results suggest that the hypophosphataemia observed was related to the metabolic process of ethanol utilizing alcohol dehydrogenase, and that acetaldehyde, a metabolite of ethanol, might induce the hyperphosphataemia in the animals.

Many reports are available about the effects of single or long term intake of ethanol on plasma calcium (Peng et al 1972; Peng & Gitelman 1974), magnesium (Victor & Wolfe 1973; Peng & Gitelman 1974) or potassium (Vetter et al 1967).

The effect of ethanol on plasma phosphate, however, seems not to have been described except by Peng et al (1972) who found no significant change in the rats after oral administration of ethanol at a dose of 2–8 g kg^{-1}. Since in our laboratory ethanol was found by chance to change the plasma phosphate level during another series of experiments, we set out to establish if there was a change of plasma phosphate level following a single administration of ethanol or acetaldehyde to the rabbits.

MATERIALS AND METHODS

Male rabbits, 1·8 to 2·3 kg were fasted overnight before experiments. The animals were separated into experimental groups as follows:

1. Oral ethanol in dose of 65 mmol kg^{-1} (3 g kg^{-1}).
2. Ethanol administration (65 mmol kg^{-1} p.o.) 1 h after 4 mmol kg^{-1} of intravenous pyrazole.
3. Disulfiram at a dose of 0·1 mmol kg^{-1} for 4 days, and oral ethanol (65 mmol kg^{-1}) 1 h after the last administration of disulfiram.
4. Acetaldehyde in dose of 1·5 mmol kg^{-1} i.v.
5. Disulfiram administration (0·1 mmol kg^{-1}) for 4 days, and 1·5 mmol kg^{-1} i.v. of acetaldehyde 1 h after the last injection of disulfiram.
6. Acetic acid administration in dose of 0·3 mmol kg^{-1} i.v.

* Correspondence.

Plasma samples were obtained by centrifugation within 15 min after the blood collection. Plasma phosphate and calcium were determined by spectrophotometric methods with the use of Iatron Pi and Ca kits. Disulfiram and pyrazole were obtained from Nakara Chemicals, Ltd and acetaldehyde was provided by E. Merck AG.

RESULTS

In the Figures, plasma phosphate is expressed as relative values of each pre-administration level designated as 100%.

Group 1. The results shown in Fig. 1 demonstrate that a single oral administration of ethanol immediately caused a hypophosphataemia followed by a

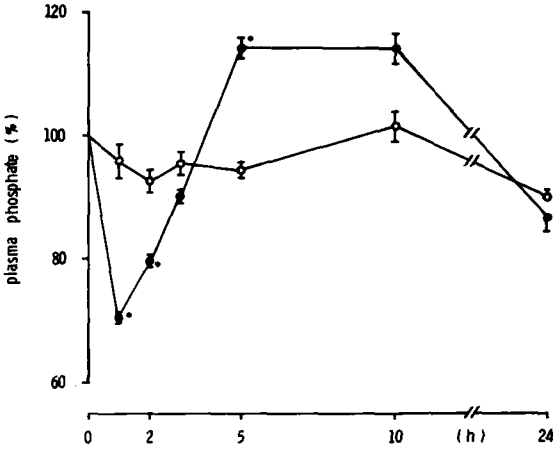

FIG. 1. Relative change of plasma phosphate in rabbits (1). Treatment: ethanol (65 mmol kg^{-1}). ●——● *experimental* (n = 7), ○——○ control (n = 4). Means and standard errors of the mean are shown. * $P < 0.01$.

hyperphosphataemia. By Student's *t*-test, the former effect was significant ($P < 0{\cdot}01$) between 1 and 2 h after administration. The latter appeared at 5 h ($P < 0{\cdot}01$) after administration and persisted for 10 h.

As shown in Table 1, the plasma calcium level decreased significantly at least for the first 5 h ($P < 0{\cdot}02$).

Group 2. Pyrazole is known to inhibit alcohol dehydrogenase (ADH). Combined administration of pyrazole and ethanol was made to see the effect on the plasma phosphate level and so to evaluate its relation to the metabolism of ethanol (Fig. 2). The

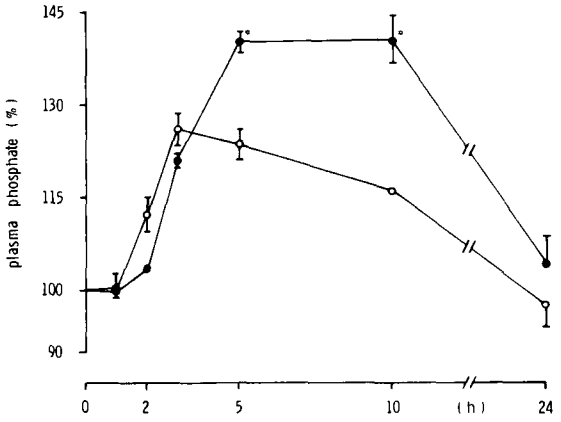

FIG. 2. Relative change of plasma phosphate in rabbits (2). Treatment: ethanol (65 mmol kg^{-1}) and pyrazole (4 mmol kg^{-1}). ●——● experimental (n = 4), ○——○ control (n = 4). * $P < 0{\cdot}05$.

same ethanol dose of 65 mmol kg^{-1} as in Group 1, with pretreatment by pyrazole, did not cause a lower level of phosphate than in the control at least for the first 2 h, but produced higher values between 5 and 10 h ($P < 0{\cdot}05$) after administration. By 24 h, plasma phosphate had returned to normal level. Plasma calcium decreased and persisted for 10 h ($P < 0{\cdot}05$) with this treatment (Table 1).

Group 3. Disulfiram, an inhibitor of acetaldehyde dehydrogenase (AldDH), was used to sustain a high level of acetaldehyde in the blood. As shown in Fig. 3, combined administration of disulfiram and ethanol had a similar effect of hypophosphataemia as with ethanol alone. However, with preadministration of disulfiram, the duration of hyperphosphataemia became prolonged, the plasma phosphate being still significantly higher than the control value ($P < 0{\cdot}05$) at 24 h after ethanol. In this group, plasma calcium decreased and persisted for 10 h ($P < 0{\cdot}02$) as in Group 2 (Table 1).

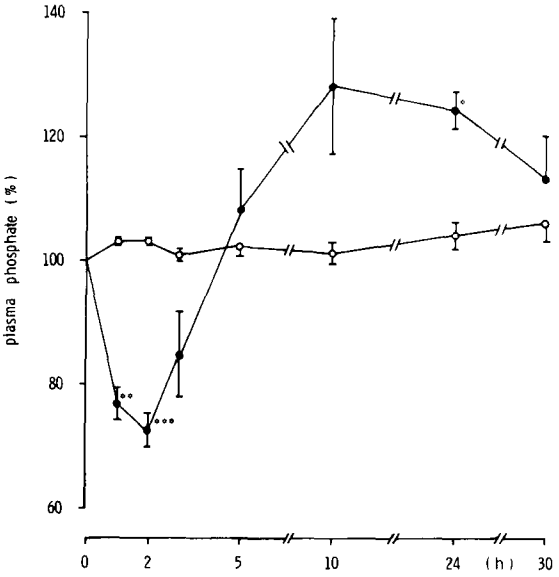

FIG. 3. Relative change of plasma phosphate in rabbits (3). Treatment: ethanol (65 mmol kg^{-1}) and disulfiram (0·1 mmol kg^{-1} × 4). ●——● experimental (n = 4), ○——○ control (n = 4). $P < 0{\cdot}05$, ** $P < 0{\cdot}02$ *** $P < 0{\cdot}01$.

Group 4. The results shown in Fig. 4 illustrate that a single injection of acetaldehyde caused a significant effect on plasma phosphate level. The level increased rapidly about up to 140% of control value as early as between 15 ($P < 0{\cdot}001$) and 30 min ($P < 0{\cdot}05$) after administration. Plasma calcium did not significantly change at any time tested.

Table 1. Effect of ethanol on plasma calcium. Plasma calcium is expressed as relative values of each pre-administration level designated as 100%. Number of animals in each group are shown in parentheses. Means and standard errors of the mean are shown.

Experimental group	1 h	2 h	3 h	5 h	10 h	24 h
Group 1 (10)	88·6 ± 1·5***	85·9 ± 1·3***	86·0 ± 2·1****	86·0 ± 1·6**	87·7 ± 1·3	91·6 ± 1·2
Control (5)	99·1 ± 2·0	96·5 ± 1·7	101·9 ± 3·0	93·0 ± 1·3	97·2 ± 3·1	95·9 ± 3·7
Group 2 (4)	88·6 ± 2·5*	88·9 ± 3·7*	88·7 ± 3·2*	85·2 ± 3·3*	84·8 ± 3·0*	94·9 ± 4·6
Control (4)	97·2 ± 2·7	97·6 ± 1·2	97·0 ± 1·9	94·6 ± 1·5	92·3 ± 0·6	89·3 ± 3·9
Group 3 (5)	91·4 ± 2·8	87·5 ± 1·7***	86·1 ± 2·0***	86·2 ± 1·2****	88·7 ± 2·2**	96·8 ± 1·5
Control (4)	96·9 ± 1·3	98·0 ± 2·0	97·1 ± 1·1	98·9 ± 1·7	97·7 ± 1·3	98·2 ± 0·7

* $P < 0{\cdot}05$, ** $P < 0{\cdot}02$, *** $P < 0{\cdot}01$, **** $P < 0{\cdot}001$.

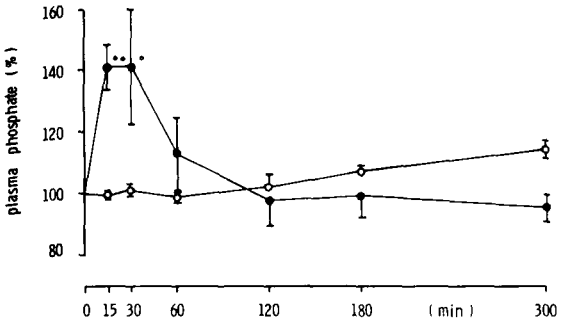

FIG. 4. Relative change of plasma phosphate in rabbits (4). Treatment: acetaldehyde (1·5 mmol kg^{-1}). ●——● experimental (n = 4), ○——○ control (n = 5). * $P < 0{\cdot}05$, ** $P < 0{\cdot}001$.

Group 5. As acetaldehyde must have disappeared rapidly from the blood in the rabbits of Group 4, the exclusive and prolonged effect of acetaldehyde without the influence of ethanol was examined in this group. Hyperphosphataemia was evident between 15 and 30 min after injection ($P < 0{\cdot}02$ and $< 0{\cdot}01$ respectively). Plasma phosphate increased quickly and reached about twice the control value (Fig. 5). Plasma calcium did not significantly change during hyperphosphataemic state.

Group 6. No significant change was observed both in plasma phosphate (Fig. 6) and calcium after administration of acetic acid, a metabolite of acetaldehyde.

DISCUSSION

Our results showed that administration of ethanol produces various changes of plasma phosphate level in rabbits. Plasma calcium, on the other hand, was not related to these changes of plasma phosphate.

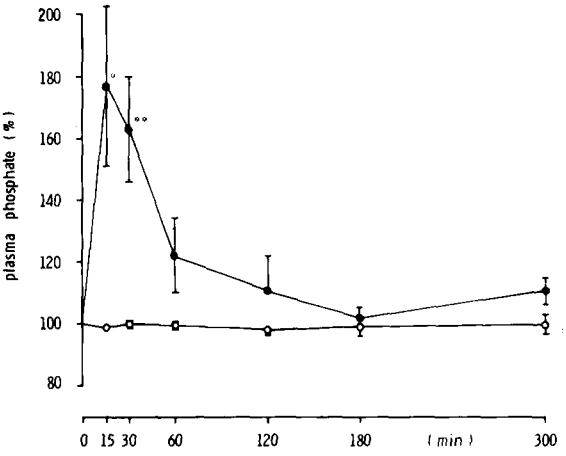

FIG. 5. Relative change of plasma phosphate in rabbits (5). Treatment: acetaldehyde (1·5 mmol kg^{-1}) and disulfiram (0·1 mmol kg^{-1} × 4). ●——● experimental (n = 5), ○——○ control (n = 5). * $P < 0{\cdot}02$, ** $P < 0{\cdot}01$.

The lack of change of blood phosphate after ethanol reported by Peng et al (1972) might be due to individual variation of phosphate level which has been known to be large among the same species.

Hypophosphataemia was observed after administration of ethanol alone or ethanol and disulfiram. When ethanol was administered with pyrazole to rabbits, however, hypophosphataemia did not appear. Thus, inhibition of ADH activity with pyrazole caused ethanol to lose its ability to cause hypophosphataemia. Plasma calcium significantly decreased in the above groups. These observations suggest that the hypophosphataemia was induced by a metabolic process of ethanol catalysed by ADH and not by ethanol itself.

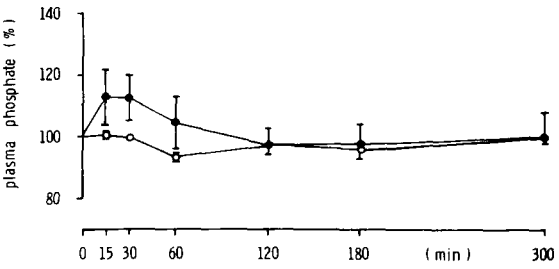

FIG. 6. Relative change of plasma phosphate in rabbits (6). Treatment: acetic acid (0·3 mmol kg^{-1}). ●——● experimental (n = 4), ○——○ control (n = 4).

When animals were pretreated with pyrazole or disulfiram, ethanol produced hyperphosphataemia, whose duration became longer especially with disulfiram. Acetaldehyde, administered intravenously, rapidly elevated plasma phosphate level, disulfiram increasing its effect. These findings suggest the hyperphosphataemia to be due to acetaldehyde, a metabolite of ethanol. The reason why plasma phosphate increased and then declined quickly after administration of acetaldehyde may be attributable to its rapid disappearance from the blood. These results also suggest that the hyperphosphataemia was produced not by metabolites nor metabolic process of acetaldehyde, but by acetaldehyde itself.

Though the mechanism by which ethanol caused hypocalcaemia has not been clarified, Peng et al (1972) suggested that the hypocalcaemia is not associated with hypophosphataemia and is not related to thyroid or parathyroid action. Also in the present study, plasma phosphate and calcium did not change in parallel. Therefore the effects of ethanol or acetaldehyde on plasma phosphate level would not be mediated by thyroid or parathyroid glands, and a different mechanism would be involved in changes of plasma calcium and phosphate due to

ethanol. Peng et al (1972) observed that urinary excretion of phosphate was significantly lowered during the 3 h following ethanol administration in rats. Though phosphate content in the urine was not examined in this study, its urinary excretion would not have produced change of plasma phosphate level after administration of ethanol or acetaldehyde.

There are some reports about creatine phosphokinase activity after ethanol administration (Lafair & Myerson 1968; Aoki 1978; Hayakawa et al 1979). According to these reports, ethanol increased plasma creatine phosphokinase, the maximum level of which appeared 8–12 h after administration. In another series of our experiments, acetaldehyde alone was also found to increase creatine phosphokinase in the plasma of rabbits (unpublished results). Therefore it is possible that the hyperphosphataemic effect due to acetaldehyde is associated with some damage of muscles that are the main phosphate reservoir in the body.

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