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COMPARISON OF THE ANTILIPEMIC EFFECT OF NICOTINIC ACID (NA) AND 3-METHYLPYRAZOLE-5-CARBOXYLIC ACID (MPC) IN RATS

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Abstract—The antilipemic effects of nicotinic acid (NA) and 3-methylpyrazole-5-carboxylic acid (MPC) were compared *in vivo* and *in vitro* in rats. Both compounds decreased significantly the levels of serum FFA and triglycerides in normal and in alloxan-diabetic rats, while MPC exerted a significant activity at much lower doses than NA. In the case of Triton-lipemia both compounds were found to lower the serum triglycerides at equivalent doses. The serum cholesterol level was decreased by NA in all tests, while MPC had effect in alloxan-diabetes only. Also *in vitro* MPC was more active on rat adipose tissue, inhibiting the lipolysis stimulated by adrenaline.

A NUMBER of agents became available in recent years which are capable of lowering the level of free fatty acids (FFA) in serum. Besides hormones, such as insulin[1] and prostaglandin,[2] numerous organic compounds of low molecular weight, such as nicotinic acid,[3, 4] salicylates,[5] isoxazole[6]- and pyrazole-derivatives,[7, 8] decrease the serum FFA significantly by inhibiting the lipolysis in the adipose tissue. Another group of agents influence the serum FFA indirectly by abolishing the effect of sympathetic discharge.[20]

Nicotinic acid is used therapeutically as a cholesterol- and triglyceride-lowering agent.[9] Carlson suggested that the decreasing effect of NA on the serum cholesterol and triglyceride levels may be due to the inhibition of FFA mobilization.[3, 4] In this paper the antilipemic effect of a 3-methylpyrazole-5-carboxylic acid (MPC), which lowers the serum FFA level effectively,[8] is compared with that of nicotinic acid (NA) in rats.

MATERIALS AND METHODS

Male Wistar Ch.B. rats weighting 210 ± 20 g maintained on a standard diet were used. The following tests were used:

(a) *Normal* rats fasted for 16 hr before the start of experiment were treated orally and decapitated 4 hr later.

(b) *Alloxan-diabetes* was produced in rats fasted for 16 hr by giving alloxan (Flukka) in a dose of 60 mg/kg i.v. Alloxan was freshly dissolved in saline and injected in a volume of 2 ml/kg .48 hr after the alloxan administration a sustained rise of blood sugar and serum lipid levels develops. The compounds tested were given at that time in two s.c. injections 3 hr apart. The animals were decapitated 6 hr after the first injection.

In this type of experiment food and water was given *ad libitum* from the time of alloxanization.

(c) *Triton-lipemia* was produced by i.v. injection of 200 mg/kg Triton WR-1339 (Serva Lab., Germany) dissolved in saline after 16 hr of fasting. The agents tested were given orally divided into two equal parts, the first part was given simultaneously with Triton, the second one 3 hr later. The rats were sacrificed 6 hr after Triton administration.

The collected blood was centrifuged immediately and the following lipid fractions of the serum were determined: FFA by the method of Dole.[1] triglycerides by Van Handel and Zilversmit[10] and total cholesterol by Zlatkis *at al.*[11]

In vitro *experiments.* The epididymal fat pads of non-fasted male rats were excised immediately after decapitation. Pieces of 170–180 mg of adipose tissue were incubated in 2 ml of Krebs–Henseleit-phosphat buffer for 60 min on 37°. Air served as gas phase. Adrenaline was given to the incubation medium at a concentration of $5{\cdot}5 \times 10^{-6}$ M. NA and MPC were added as well at different concentrations. The stopping of the incubation was accomplished by the addition of 10 ml of Dole-mixture. After homogenization the FFA content was determined according to Dole.[1]

The NA as its Na-salt (Serva Lab., Germany) was dissolved in distilled water or in saline. The MPC was first dissolved in 5% (w/v) $NaHCO_3$ and diluted later with saline or water. The amount needed from the agents tested was made up to 5 mg/kg body wt. with water in the case of oral treatment and to 2 ml/kg with saline when given s.c.

The statistical evaluation of the results was carried out by the *t*-test.

RESULTS

Effect on serum FFA (*Fig.* 1). Both NA and MPC were found to decrease significantly the level of serum FFA in normal as well as in alloxan-diabetic rats. The dose-response curves show the following characteristics: (a) MPC is more active than NA, especially, when the smallest doses, still capable to induce a significant effect, are compared. (b) In the case of both agents the effect is dose-dependent. (c) The slope of the dose-response curve of NA is steeper than that of MPC. (d) Lower doses of NA can increase the serum FFA level in normal rats.

Effect on serum triglycerides (*Fig.* 2). Both compounds were found to decrease the level of serum triglycerides to a considerable extent. The dose-response curves can be characterized as follows: (a) MPC is more active than NA in normal and alloxan-diabetic states, especially, when the smallest doses, which still induce a significant effect, are compared. On the contrary, in Triton-lipemia significant effect is produced by equivalent doses (100–400 mg/kg). (b) The effect of both agents is dose-dependent. (c) The slope of the dose-response curve of NA—similar to the findings obtained in the case of FFA—is steeper than after MPC.

Effect on serum cholesterol (*Fig.* 3). NA and MPC exerted different effects on the serum cholesterol level: NA had a decreasing effect in all three tests, while MPC showed activity only in alloxan-diabetic animals. In this last test MPC seemed to be more effective than NA and its effect was dose-dependent as well.

In vitro *experiments* (*Fig.* 4). In rat epididymal adipose tissue both NA and MPC inhibited the accumulation of FFA caused by adrenaline. In this test MPC was found

to be more active than NA again; while the smallest but still effective concentration of NA was found to be in the range of 10^{-6} M, that of MPC of 10^{-7} M only.

DISCUSSION

It seems from the above results that MPC can influence the lipid metabolism in rats at much lower doses than NA. In accordance with previous reports[12, 13] MPC can decrease the serum FFA as well as the triglyceride levels in normal rats. In alloxan-diabetes also NA can effectively reduce the serum FFA[14, 15] and triglyceride levels.[14] In this test MPC proved to be more active than NA again.

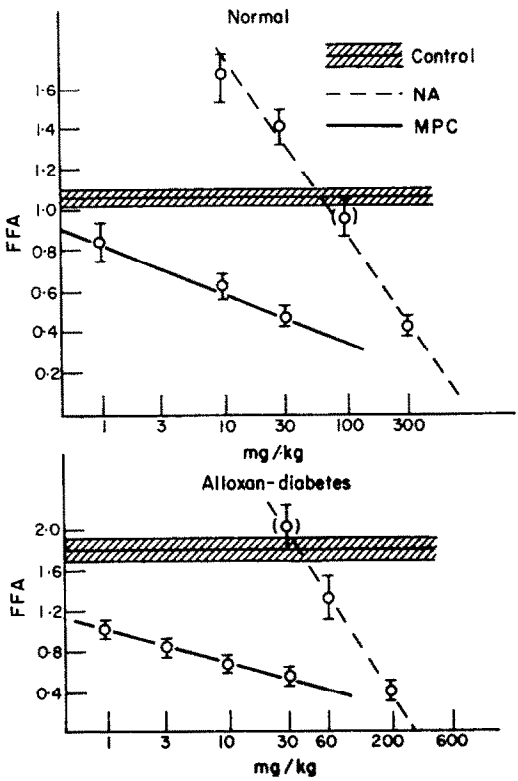

FIG. 1. The effect of MPC and NA on the level of serum FFA in normal and alloxan-diabetic rats. (Serum FFA in mEquiv/l. Results are given as the mean values ± S.E.M. of 8–15 individual data. Values in brackets are not significantly different from controls: $P > 0{\cdot}05$.).

The decreasing effect of MPC and NA on the serum FFA and triglyceride levels may have a common basis: the inhibition of lipolysis in adipose tissue. The NA was shown to inhibit the lipolysis stimulated by catecholamines on rat adipose tissue *in vitro*.[16, 17] These observations were confirmed and the minimal effective concentration of NA was found to be in the range of 10^{-6} M. MPC was shown to decrease the spontaneous lipolysis.[8] while we found that the adrenalin-stimulated lipolysis can be inhibited as well. *In vitro* MPC had an effect at lower concentration than NA. These *in vitro* observations are in correlation with the changes of serum FFA *in vivo*. The decrease of the serum triglyceride as well as FFA levels is consistent with the view,

that serum FFA level has a dominant role in the regulation of the synthesis of serum triglycerides.[18] It seems likely that the reduction of serum FFA level is causally related to the decrease of triglycerides in alloxan-diabetes as well,

The similarity in action of MPC and NA appears to be inconsistent with the facts, that (a) the dose-response curves after MPC and NA (Fig. 1 and 2) are not parallel

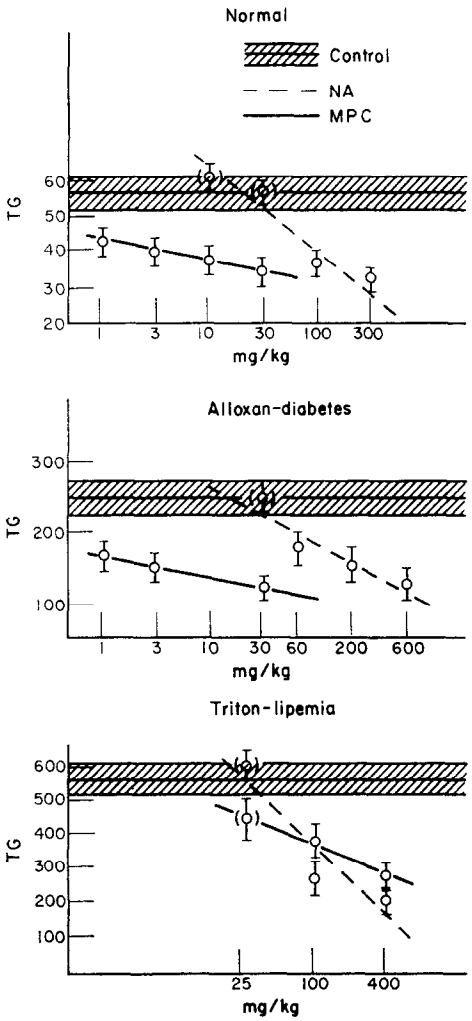

FIG. 2. The effect of MPC and NA on the level of serum triglycerides (TG) in normal, alloxan-diabetic and Triton-lipemic rats. (Serum triglycerides in mg/100 ml of serum. For further details see Fig. 1).

and (b) low doses of NA, unlike MPC, elevate the serum FFA level. The lack of similarity of dose-response curves may be explained by different metabolism of the two agents and not necessarily by their different mode of action. Pereira[19] found an elevation of the serum FFA level in rats after NA depending on the dose applied

and on the interval after the administration. In Triton-lipemia significantly higher doses of MPC were needed compared to those applied in normal and alloxan-diabetic animals to produce a significant decrease of serum triglyceride and cholesterol levels. Beside a decrease of serum FFA other mechanisms may be responsible for the reduction of serum triglycerides in Triton-lipemia after MPC administration.[13]

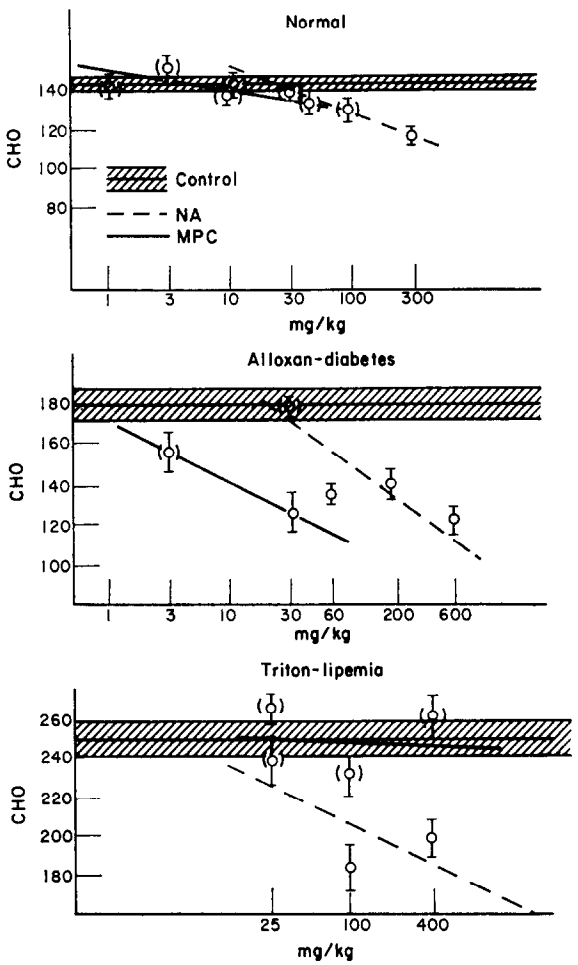

FIG. 3. The effect of MPC and NA on the level of serum cholesterol (CHO) in normal, alloxan-diabetic and Triton-lipemic rats. (Serum cholesterol in mg/100 ml of serum. For further details see Fig. 1).

The serum cholesterol level is effected differently by NA and MPC. The NA—though to a different extent—decreased the serum cholesterol in all three tests, while MPC had an activity in alloxan-diabetes only. Taking into account the marked effect of both compounds on the serum FFA level, the inhibition of FFA mobilization may have no determining role in controlling the serum cholesterol level as has been suggested for NA.[3, 4] In alloxan-diabetes the serum FFA level seems to play some role

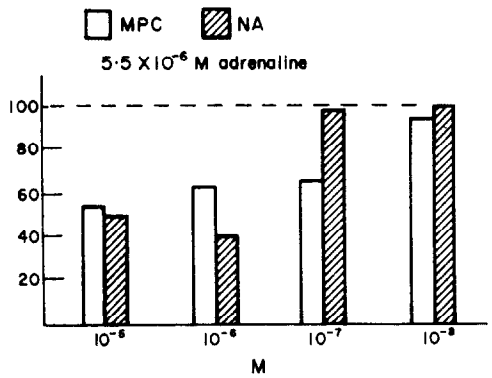

FIG. 4. The effect of MPC and NA on the lipolysis stimulated by adrenaline in rat epididymal adipose tissue. (The effect of adrenaline is taken as 100 per cent .The columns show the effect of adrenaline in the presence of agents tested as the mean of 4–5 individual data).

in controlling the serum cholesterol, perhaps, by determining the amount of acetyl-CoA available for cholesterol biosynthesis in liver.

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