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SHORT COMMUNICATIONS

Additive effect of combined spironolactone and phenobarbital treatment on hepatic bilirubin UDP-glucuronyltransferase

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It is known that the administration of phenobarbital (PB) or spironolactone (SP) to rats accelerates plasmatic bilirubin clearance and increases the bilirubin diglucuronide to monoglucuronide excretion ratio, probably by increasing hepatic bilirubin UDP-glucuronyltransferase (UDP-GT) activity [1–5]. Such effects were observed with PB daily doses of 3–125 mg/kg body wt (12–492 μmol/kg body wt), i.p. [1, 2] and SP daily doses of 100 mg/kg body wt (240 μmol/kg body wt), i.p. [3–5].

Although the dose–response effect of PB on hepatic microsomal bilirubin UDP-GT has been reported previously [2], no study has examined the dose–response curve of SP on that system. In addition, the effect of PB and SP, when administered together, on microsomal cytochrome P-450 dependent monooxygenases has also been investigated [6], but the effect of such a treatment on bilirubin UDP-GT remains unknown.

Therefore, in this study we examined the effects of different doses of SP on bilirubin UDP-GT of rat liver microsomes in comparison with the effects produced by equimolecular doses of PB. The effect produced by both inducers on the enzyme system, when they were given in combination, was also investigated.

Materials and methods

Animals. Male Wistar rats (250–320 g) were used. All animals were housed in a 22° temperature-controlled room with alternating 12-hr light/dark cycles for at least 1 week prior to the study. The animals were maintained *ad lib*. on a standard laboratory pellet diet and were allowed free access to water and saline solution during treatment.

Experimental procedures and assays. A group of rats was injected with PB (sodium salt) i.p., given as a daily dose of 25–395 μmol/kg body wt, dissolved in 0.9% NaCl solution, for 3 consecutive days prior to the experiment. Another group received SP in the same way but as a daily dose of 25–240 μmol/kg body wt, dissolved in propyleneglycol. A third group of animals received both inducers simultaneously and in the same way, in the relationships 100 to 25, 150 to 50, 25 to 100, and 50 to 150 μmol/kg body wt/day of SP and PB respectively. A fourth group of rats was injected with 0.9% NaCl solution or propyleneglycol and was used as the control.

The livers were perfused *in situ* with iced 0.9% NaCl solution through the portal vein 24 hr after the last injection. Then they were excised, blotted on filter paper, and weighed. The liver–body wt ratio was calculated for each animal. Hepatic microsomal suspensions were prepared in 250 mM sucrose, 1 mM EDTA as described [7], and the bilirubin UDP-GT activity was determined [8]. Total protein in liver microsomal preparations was also determined [9].

Statistical analysis. The results are expressed as mean values ± SEM. Student's *t*-test was used for comparison of the data. $P < 0.05$ or less was considered to be statistically significant.

Chemicals. All the chemicals used were of reagent grade quality. Bilirubin, UDPGA, and SP were from the Sigma Chemical Co. (U.S.A.).

Results

The liver–body wt ratio and hepatic bilirubin UDP-GT activity did not show differences in control rats that were injected with saline solution or propyleneglycol.

The effects produced by PB or SP administration on the liver–body wt ratio are presented in Fig. 1. PB produced a significant increase in the ratio at all doses tested, whereas SP produced such an effect only at high doses. When we measured bilirubin UDP-GT activity of liver microsomes, a significant inducer effect was produced by PB at doses over 100 μmol/kg body wt, whereas SP was effective at all doses tested. The comparison of the inducer effects of both compounds on the enzyme activity showed that SP was about two to three times more effective than PB. Moreover, a maximal bilirubin glucuronidation rate was reached with PB doses over 200 μmol/kg body wt. In contrast, a maximal rate of bilirubin glucuronidation was not obtained with SP within the range of doses tested (Fig. 2).

The simultaneous administration of SP and PB in the relationships 100 to 25, 150 to 50, 25 to 100 and 50 to 150 μmol/kg body wt/day increased significantly the liver–body wt ratio (3.6 ± 0.1, 3.7 ± 0.1, 3.7 ± 0.1 and 3.9 ± 0.2 respectively) in comparison with control values. This combined treatment also increased bilirubin UDP-GT activity, but percent increases over control values did not differ from the increases calculated from the sum of the respective individual effects (Fig. 3).

Discussion

In this study, compounds that are known to increase bilirubin UDP-GT were administered to adult male rats, alone or in combination, for comparison of their effects and to determine whether they were additive.

The results obtained clearly demonstrated that SP was more effective than PB as an inducer of rat liver bilirubin UDP-GT. With the lowest SP dose used, the increase in

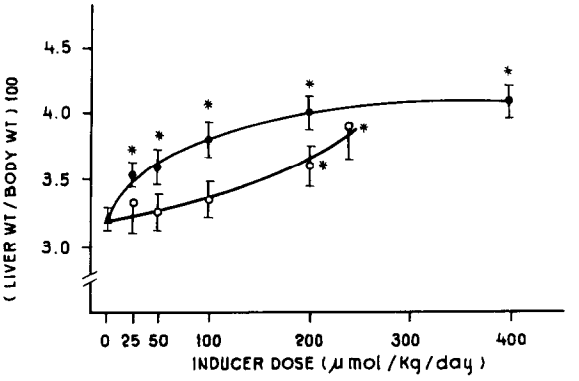

Fig. 1. Effects of PB (●—●) and SP (○—○) treatments on liver–body wt ratio. Each point is the mean value ± SEM obtained from three to six rats. Key: (*) significantly different from control animals (▲).

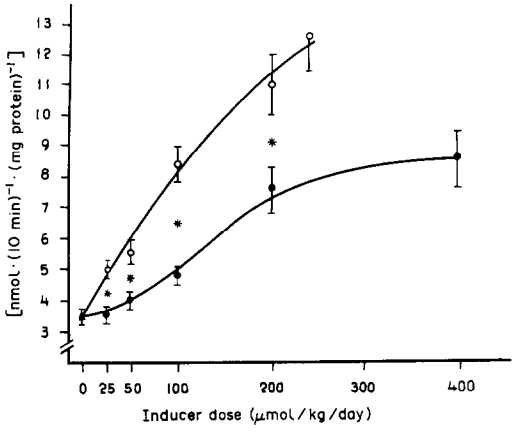

Fig. 2. Effects of PB (●—●) and SP (○—○) treatments on hepatic bilirubin UDP-GT activity. Values are expressed as nmol of total bilirubin conjugated per 10 min per mg of protein. Each point represents the mean value ± SEM obtained from three to six different microsomal preparations. The triangle corresponds to the mean value for the control. Key: (*) statistically significant differences between groups.

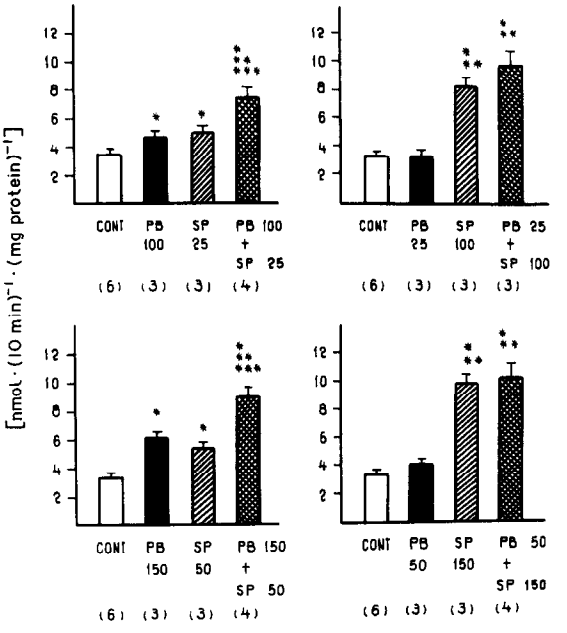

Fig. 3. Effects of PB and SP alone or in combination on hepatic microsomal bilirubin UDP-GT activity. PB and SP were administered as daily doses of 25, 50, 100 and 150 μmol/kg body wt, i.p., for 3 consecutive days prior to the experiment. The bars represent mean values ± SEM. The number of animals is given in parentheses. Key: (*) significantly different from controls; (**) significantly different from PB alone; and (***) significantly different from SP alone.

enzyme activity was 42% over control values. Such an effect could be reached by PB only at doses four to five times higher (see Fig. 2). A greater inducer effect of SP was also observed for the dose commonly used for experimental purposes [10–12]. We could speculate that SP or its metabolite, canrenone [13, 14], may be more capable than PB of binding to a receptor or that the resultant complex is more effective in initiating gene expression in the nucleus [15].

Since low doses of SP did not change the liver–body wt ratio (see Fig. 1), we can assume that the steroid not only was more effective than PB as an inducer of bilirubin UDP-GT but also was more specific.

The effects produced by conjoint treatment with SP and PB on UDP-GT appeared to be additive. This is in contrast to the effects produced on several microsomal cytochrome P-450 dependent monooxygenases when SP and PB were administered together [6]. Despite the fact that one cannot rule out the possibility that the two compounds interact with the same induction receptor, it seems more likely that SP and PB (due to their different molecular structures and properties) interact with different receptors, and the complexes thus formed affect the genetic machinery of the cell differently to induce the synthesis of catalytic units. Therefore, the effects on UDP-GT observed when SP and PB were administered together appear to be the result of the sum of individual inductive properties. Moreover, the effect of combined treatment on liver–body weight ratios was in accordance with the effects produced by SP and PB when administered alone.

In summary, we observed that SP was a more effective and specific inducer than PB on liver microsomal bilirubin UDP-GT. The combined effect of the two compounds acting simultaneously was equal to that expected by the sum of individual effects, suggesting that they could function via independent mechanisms.

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Nimodipine reduces postischemic lactate levels in the isolated perfused rat brain

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During recent years, several reports have appeared demonstrating improvements of postischemic brain energy metabolism by calcium antagonists [1–6]. The data include increases in the levels of high-energy phosphates, glucose, glucose-6-phosphate, and decreases in lactate levels. The faster restitution of high-energy phosphates was demonstrated for a variety of calcium antagonists including dihydropyridine, benzothiazepine, and phenylalkylamine derivatives. As shown for emopamil, this effect is due to the vasodilating properties of the calcium antagonists [5]. The faster postischemic degradation of lactate by emopamil, however, was unrelated to flow changes suggesting a different site of action. Besides their properties to block slow inward calcium current, the phenylalkylamines possess a variety of other pharmacological effects unrelated to calcium antagonism [7]. Gallopamil, for instance, is a potent 5-HT2 antagonist in a dose range similar to its ability to bind to the L-type calcium channel [8]. The dihydropyridine derivative nimodipine has not been shown to have such action at 5-HT2 receptors and compared to phenylalkylamine calcium antagonists reveals a somewhat different profile of nonspecific side effects. The present investigation was set up to determine whether nimodipine also faster reduces postischemic brain lactate levels. Such action would provide support to the concept that the previously monitored changes in cortical lactate are related to calcium entry blockade.

Materials and methods

Materials. Nimodipine was a gift from Bayer (Leverkusen, F.R.G.). Enzymes, coenzymes and substrates were obtained from Boehringer (Mannheim, F.R.G.) DL-dithiothreitol. HEPES (4-(2-hydroxyethyl)-piperazine-1-ethane-sulfonic acid), imidazole, 2-mercaptoethanol, and 1-thioglycerol were purchased from Sigma Chemical Co (St Louis, MO). All other chemicals were of reagent grade. Male Sprague–Dawley rats (IWV, Geretsried, F.R.G.) weighing 200–230 g were used. The animals were maintained under controlled environmental conditions (12 hr dark/light cycle, 23 ± 1°, 55 ± 5% rel. humidity) and kept on standard diet (Altromin, Lage, F.R.G.) and tap water *ad lib.* until starting the experiment.

Perfusion technique. The rat brain was isolated [9] and perfused with a fluorocarbon (FC 43) emulsion (Green Cross Corp., Osaka, Japan) following a technique described previously in detail [10]. Perfusion pressure (100 mmHg) was maintained at a constant level. Bipolar electroencephalograms were recorded from the parietal region of each hemisphere (EEG preamplifier BioAC; Hellige, Freiburg, F.R.G.). Nimodipine was added to the perfusion medium at a concentration of 0.5 μmol/l before perfusion was started. After a perfusion period of 30 min, ischemia was induced for 30 min by switching off the medium supply. Following ischemia perfusion was reinstituted for 5, 15, 25, or 40 min. Flow rate was determined by collecting venous perfusate over one min by means of a burette. Metabolism was stopped by immersing brains into liquid nitrogen [11].

Enzymatic methods. Cerebral cortical tissue was removed from the brain, weighed and extracted at −20° [12]. Creatine-phosphate, ATP, ADP, AMP, glucose, pyruvate and lactate were measured spectrophotometrically [13]. Glucose-6-phosphate and fructose-1,6-bisphosphate were measured fluorimetrically [14]. The energy charge of the adenylate pool EC = ATP + 0.5 ADP/ATP + ADP + AMP was calculated according to Atkinson [15].

Statistics. Values are presented as means ± SD. For the analysis of the influence of nimodipine on postischemic lactate levels and on perfusion rate, two-way analysis of variance was used. The rate of postischemic lactate degradation was calculated as the difference between the respective lactate level and the mean value at the end of ischemia divided by the respective reperfusion time. Only data from 5, 15 and 25 min of recirculation were included since lactate degradation seemed to be nearly linear during this time span. Lactate degradation rates of control and nimodipine-treated brains were compared with Student's *t*-test after releasing two values by means of the Dixon test. The influence of mean flow rate on the rate of lactate degradation was determined by linear regression analysis and *F*-test.

Results

Cerebral flow rate was unchanged by nimodipine during preischemic perfusion. During postischemic recirculation, however, the calcium antagonist caused an increase in perfusion rate (Fig. 1). Cortical high-energy phosphates and glycolytic intermediates were determined after 30 min of perfusion, 2 and 30 min of complete global ischemia. No change in cortical metabolite levels was induced by nimodipine during control perfusion and ischemia (data not shown; for lactate levels see Fig. 2). After 2 min of ischemia, energy metabolism was not different from the corresponding controls in the nimodipine-treated group (data not shown; for lactate levels see Fig. 2). During recirculation, treatment with the calcium antagonist led to lower lactate levels (Fig. 2). The rate of lactate degradation was significantly higher in the nimodipine-treated groups (0.30 ± 0.04 μmol/g/min in the control group vs 0.36 ± 0.04 μmol/g/min in the nimodipine group; $P < 0.01$) ADP and glucose-6-phosphate levels were also reduced by the calcium antagonist during postischemic reperfusion (data not shown). In Fig. 3 the rates of lactate degradation vs mean postischemic flow rates are depicted for control and nimodipine-treated brains. Linear regression analysis revealed no significant correlation between both parameters.

Discussion

In accordance with our results, nimodipine has been consistently shown to improve the postischemic hypoperfusion state [16–19]. Locally performed flow determinations indicate that this improvement in postischemic blood flow by nimodipine is regionally heterogeneous [19]. In an earlier study, we reported on improved high-energy