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# Comparative Hypoglycemic and Hypoketonemic Effects of Tolbutamide and Glypentide in Rat *

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Fed and 24 h fasted rats were treated by stomach tube with different doses of either tolbutamide or glypentide and they were compared with controls treated with placebo. At low doses glypentide was ten times more effective as hypoglycemic agent than tolbultamide whereas it was only twice as effective in the fasted rats. Supramaximal doses of either drug produced the same effect decreasing blood glucose levels. Both drugs were able to decrease the rise of blood ketones in fasted rats, but the comparative effect was not parallel to the one observed on glycemia and not proportional to the doses used. The different responses are interpreted as function of the hypoglycemic effect, which would be mainly mediated through the insulinotropic action of these drugs, while the hypoketonemic would be the result of both their insulinotropic effect and their direct action on lipolysis and ketogenesis.

Sulfonylureas are oral hypoglycemic agents currently being used in the treatment of maturity-onset diabetes (2, 6, 16). Tolbutamide is the most widely used sulfonylurea both for laboratory and clinical experiments, but a great number of other sulfonylureas with different hypoglycemic activity have been developed. Some of them, recently discovered, are particularly potent (15). This is the case with glypentide, whose structure is N-4-beta-(o-anisamidethyl)-benzene-sulphonyl-N′ cyclopentylcarbamide (17), and has been shown to be about 30 times more potent as hypoglycemic agent in rabbits than tolbutamide (11, 17). As there are no available comparative data between the hypoglycemic effects of tolbutamide and glypentide in the rat, in the present work we have studied this point. It has been

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shown that the sulfonylureas have antiketonemic effects which migth be related to their antilipolytic actions (4, 5). As it was recently shown that the *in vitro* antilipolytic effect of glypentide is greater than that of tolbutamide (12, 13), this work was extented to determine the effect of these drugs on the level of blood ketone bodies in the fasted rat.

## Materials and Methods

Female rats of the Sprague-Dawley strain weighing between 160-190 g were used. Half of the animals were fasted for 24 h before the administration of the drug and were maintained on this dietary condition for the whole experiment. The other rats were fed *ad libitum* with rat chow. Both fed and fasted rats were divided in three groups: one receiving different doses of tolbutamide suspended in 0.5 % carboxymethyl-cellulose containing Tween-80 (0.25 %), another receiving different doses of glypentide in the same medium and a third group receiving only the medium. All the treatments were administered as a single dose by stomach tube, without anesthesia. Blood was collected drop-wise from the cut tip of the tail into heparinized porcelain plates immediately before and exactly 1, 3, 6 and 24 h after the treatment. Protein-free supernatans (1:10) were prepared with $Ba(OH)_2$-$ZnSO_4$ (19) and analyzed for glucose with glucose oxidase (14). In the samples coming from fasted animals, total ketones were also analyzed (3). The results have been expressed for each rat as the percentage of the values observed at 0 time.

## Results

The treatment with a single dose of either tolbutamide or glypentide to rats by stomach tube produced a decrease in the blood glucose levels that depended on the doses and on the dietary status of the animals. In the fed animals (fig. 1), the lower doses of drugs that produced a significant hypoglycemic response were

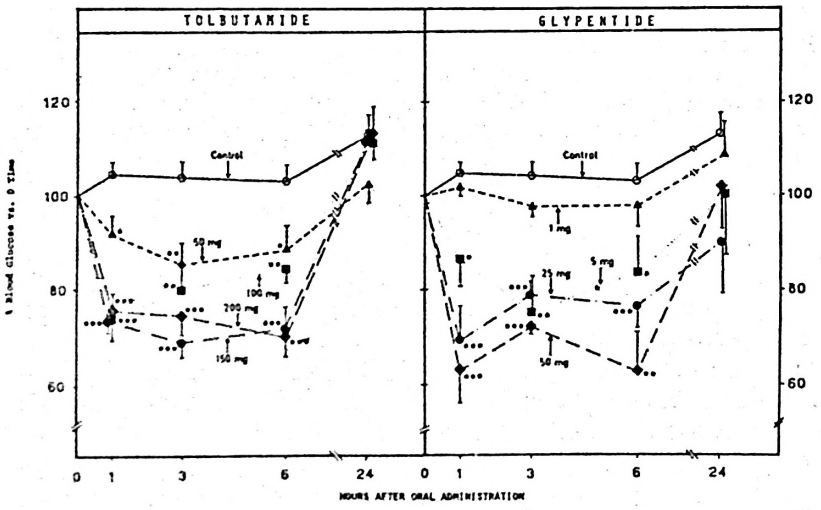


Fig. 1. *Effect of different doses of tolbutamide and glypentide on blood glucose levels in the fed rat.*

Doses correspond to mg/kg body weigth. Values are expressed as mean ± S.E.M. of 5-8 rats per group. Asterisks correspond to the statistical significativities of each group versus the controls: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

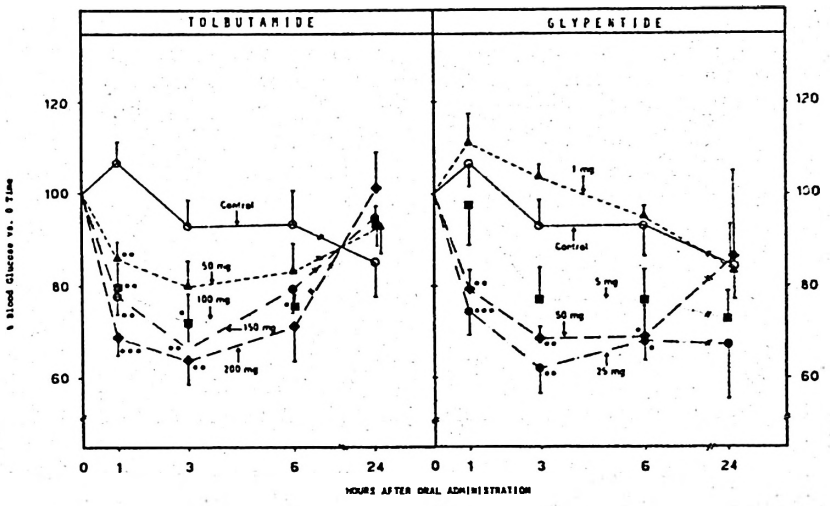


Fig. 2. *Effect of different doses of tolbutamide and glypentide on blood glucose levels in the fasted rat.*

Doses correspond to mg/kg body weigth. Values are expressed as mean ± S.E.M. of 5-8 rats per group. Asterisks correspond to the statistical significativities of each group versus the controls: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

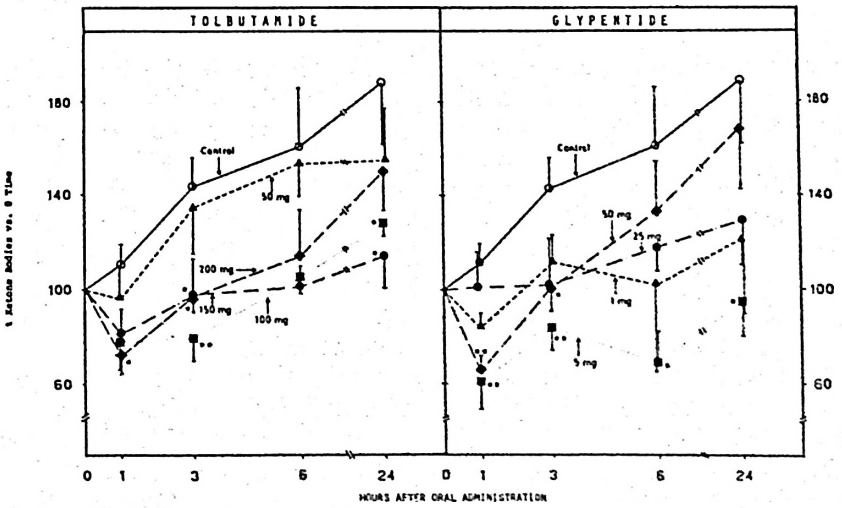


Fig. 3. *Effect of different doses of tolbutamide and glypentide on blood total ketone body levels in the fasted rat.*

Doses correspond to mg/kg body weigth. Values are expressed as mean ± S.E.M. of 5-8 rats per group. Asterisks correspond to the statistical significativities of each group versus the controls: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

50 mg/kg of body weigth of tolbutamide and 5 mg/kg of glypentide. The effects of these doses of both drugs were fully comparable. In either case the effect was significant after 1 h of the treatment and was maintained up to 6 h, while it disappeared after 24 h. Higher doses of the drugs had correspondingly greater effects up to a certain level as doses above 150 mg/kg of tolbutamide and 25 mg/kg of glypentide did not produce any further hypoglycemic effects.

Although the changes observed with the administration of these drugs to fasted animals were qualitatively very similar to those observed in the fed animals, the actual hypoglycemic activities were smaller in the fasted rats (fig. 2). The effect of 50 mg/kg of tolbutamide was only significant 1 h after the treatment, while thereafter the values did not differ from the control ones. In the fasted rats the effect of glypentide with doses of 5 mg/kg was not observed, while 25 mg/kg had a very powerful and significant hypoglycemic effect.

The hypoglycemic activity of these drugs in the fasted rat did not follow their hypoketonemic effects. Doses of 50 mg/kg of tolbutamide did not affect the rise in blood ketones in fasted animals (fig. 3). Higher doses of tolbutamide produced a significant reduction in this parameter which is not directly proportional to the doses used. With glypentide, doses of 5 mg/kg, which did not affect the blood glucose levels, produced an intense and significant hypoketonemic effect even after 24 h of the treatment. Higher doses of glypentide show smaller effects on these parameters (fig. 3). This biphasic picture again did not parallel the dose-response change of glycemia.

## Discussion

Tolbutamide has been considered a first generation sulfonylurea (15), while glypentide would correspond to the second generation, having a greater hypoglycemic effect. In the present study it is seen that doses of about one tenth of glypentide produce the same hypoglycemic effect as tolbutamide in the fed rat. This difference in activity between both drugs is smaller than that observed in rabbits (11), where the equihypoglycemic doses of both drugs differ by a factor of thirty. The different comparative activity between both drugs is also observed in the same species but under different dietary conditions as it is shown here that the equihy poglycemic doses of both drugs differ only by a factor of two in the fasted rat. As the hypoglycemic effect of sulfonylureas can be correlated with their insulinotropic action on the pancreas (9), these different responses with species and with dietary conditions may be the consequence of the different sensitivity of the beta cell to response to these stimulus. Actually, it is well known that the same stimulus produces very different responses in the rate of insulin release by the pancreas of rats and rabbits (10, 18) and fed and fasted animals (7, 8, 21, 22).

The hypoketonemic effect of the two sulfonylureas in the fasted rat is not correlated with their hypoglycemic effect with regard to the doses. Alterations in the blood level of ketones seem to be controlled by the composite rate at which free fatty acids are released from the peripheral adipose tissues, transported to the liver and there being used as substrates for ketogenesis, and also by the rate at which they are utilized by extrahepatic tissues. All these pathways are affected by insulin. Thus, the effect of sulfonylureas on blood ketones must be influenced by their effect on insulin release by the pancreas. On the other hand it is known that these drugs directly affect different sites of ketone body metabolism, including inhibition of liver ketogenesis (4, 5) and of adipose tissue lipolysis (1, 12, 13, 20). As the mixture of all these factors migth be acting at once in the

effect of these drugs on blood ketones, it is not surprising that their sensitivity on this parameter is quite different from that on blood glucose.

## Resumen

A ratas hembras alimentadas y ayunadas de 24 horas, se les suministró por sonda estomacal diferentes dosis de tolbutamida o de glipentida y se compararon con ratas controles, a las que se les suministró el placebo. Para dosis bajas, la glipentida es diez veces más efectiva como agente hipoglucemiante que la tolbutamida en las ratas alimentadas, mientras que en las ayunadas, solamente es el doble. Las dosis supramáximas de ambas drogas tienen igual efecto hipoglucemiante. Ambas drogas son capaces de disminuir la cetonemia de las ratas ayunadas, pero el efecto comparativo no es paralelo al observado sobre la glucemia y no es proporcional a las dosis empleadas. Las diferentes respuestas se interpretan en función del efecto hipoglucemiante, debido principalmente a la acción insulinotrópica de estas drogas, mientras que el efecto hipocetonémico sería el resultado de su efecto insulinotrópico y de su acción directa sobre la lipolisis y sobre la cetogénesis.

## References

1. ALLEN, D. O., LARGIS, E. E. and ASHMORE, J.: *Diabetes*, **23**, 51-54, 1974.
2. BERTRAM, F., BENDFELDT, E. and OTTO, H.: *Dtsch. med. Wschr.*, **80**, 1455-1460, 1955.
3. BESSMAN, S. P. and ANDERSON, M.: *Fed. Proc.*, **16**, 154, 1957.
4. BEWSHER, P. D., MAYHEW, D. and ASHMORE, J.: In «Tolbutamide... after ten years» (Butterfield, W. J. H. and Van Westering, W., eds.), Excerpta Medica Foundation. Amsterdam, 1967, pp. 208-214.
5. BOSHELL, B. R., ZAHND, G. R. and RENOLD, A. E.: *Metabolism*, **9**, 21-29, 1960.
6. BUTTERFIELD, W. J. H. and VAN WESTERING, W.: In «Tolbutamide... after ten years», Excerpta Medica Foundation, Amsterdam, 1967.
7. FELDMAN, J. M. and LEBOVITZ, H. E.: *Endocrinology*, **86**, 313-321, 1970.
8. FELDMAN, J. M. and LEBOVITZ, H. E.: *Endocrinology*, **92**, 1469-1474, 1973.
9. GANDA, O. P., KAHN, C. B., SOELDNER, J. S. and GLEASON, R. E.: *Diabetes*, **24**, 354-361, 1975.
10. GARCÍA-HERMIDA, O. and GÓMEZ-ACEBO, J.: *Biochem. Biophys. Res. Commun.*, **57**, 209-215, 1974.
11. GARCÍA-RAFANELL, J. and MORELL-MESTRE, J.: *Rev. esp. Fisiol.*, **30**, 91-69, 1974.
12. HERRERA, E.: *Life Sci*, **16**, 645-650, 1975.
13. HERRERA, E.: *Acta Diabetol. Lat.*, **12**, 106-113, 1975.
14. HUGGETT, A. St. G. and NIXON, D. A.: *Lancet*, **2**, 368-370, 1957.
15. LOUBATIERES, A., MARIANI, M. M., RIBES, G. and ALRIC, R.: *Acta Diabetol. Lat.*, **10**, 261-282, 1973.
16. MEHNERT, H.: *Diabetes*, **11**, 80-86, 1962.
17. MORELL, J.: *Biochem. Pharmacol.*, **23**, 2922-2924, 1974.
18. PENTO, J. T., KAGAN, A. y GLICK, S. M.: *Horm. Metab. Res.*, **6**, 177-180, 1974.
19. SOMOGY, M.: *J. Biol. Chem.*, **160**, 69-73, 1945.
20. STONE, D. B., BROW, J. D. and COX, C. P.: *Amer. J. Physiol.*, **210**, 26-30, 1966.
21. TURNER, D. S. and YOUNG, D. A. B.: *Acta Endocr.*, **72**, 46-53, 1973.
22. VOYLES, N., GUTMAN, R. A., SELAWRY, H., FINK, G., PENHOS, J. C. and RECANT, L.: *Hormone Res.*, **4**, 65-73, 1973.