Studies on the Site of Action of Tolbutamide in the Lipolytic System

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Summary. The antilipolytic effect of tolbutamide was studied under different conditions in isolated rat adipose tissue, using norepinephrine, theophylline and dibutyl 3',5'-AMP as lipolysis-stimulating agents. A selective blockade was performed with alpha and beta-blocking agents (phentolamine and Kö 592) to investigate a possible relation of tolbutamide to the adrenoceptive structures of the fat cell. — 1. Tolbutamide exerts an antilipolytic effect on norepinephrine and theophylline-stimulated lipolysis in two different concentration ranges. — 2. When using dibutyl 3',5'-AMP, this biphasic pattern disappears. — 3. Tolbutamide shows an additional antilipolytic activity in adipose tissue pretreated with the beta blocker Kö 592, whereas this effect could not be obtained after pretreatment with the alpha blocker phentolamine. — From the results obtained the conclusion is drawn that tolbutamide, like phentolamine, at least in concentrations ranging from 12.5 to 50 µg/ml, influences a reaction subsequent to the formation of cyclic 3',5'-AMP.

Etude sur le site d'action du tolbutamide dans le système lipolytique

Résumé. L'effet antilipolytique du tolbutamide a été examiné dans différentes conditions à l'aide du tissu adipeux isolé du rat. Pour la stimulation de la lipolyse, on a employé de la noradrénaline, de la théophylline et du dibutyl 3',5'-AMP. Pour découvrir une relation possible entre le tolbutamide et les structures adrénocaptives de la cellule adipeuse, on a fait un blocage sélectif avec des agents bloquant les récepteurs alpha et bêta (phentolamine et Kö 592). — 1. Le tolbutamide a un effet antilipolytique, qui agit dans deux zones différentes de concentration, sur la lipolyse stimulée par la noradrénaline et la théophylline. — 2. En utilisant le dibutyl 3',5'-AMP ce modèle biphasique n'existe pas. — 3. Dans le

tissu prétraité avec le bêta bloquant, Kö 592, le tolbutamide a un effet antilipolytique supplémentaire; dans le tissu prétraité avec l'alpha bloquant, phentolamine, cet effet ne se produit pas. — Ces résultats mènent à la conclusion que le tolbutamide, au moins dans une zone de concentration de 12,5–50 µg/ml, agit sur une réaction après la formation de 3',5'-AMP cyclique.

Untersuchungen zum Angriffspunkt des Tolbutamid im lipolytischen System

Zusammenfassung. Der antilipolytische Effekt von Tolbutamid wurde unter verschiedenen Bedingungen am isolierten Rattenfettgewebe untersucht. Als Stimulatoren der Lipolyse wurden Nordrenalin, Theophyllin und Dibutyl 3',5'-AMP verwendet. Um eine mögliche Verbindung von Tolbutamid mit den adrenoceptiven Strukturen der Fettzelle herauszufinden, wurde eine selektive Blockade mit alpha- und beta-blockierenden Substanzen (Phentolamin und Kö 592) durchgeführt. — 1. Tolbutamid wirkt in zwei verschiedenen Konzentrationsbereichen auf die Noradrenalin- und Theophyllin-stimulierte Lipolyse antilipolytisch. — 2. Bei Verwendung von Dibutyl 3',5'-AMP geht dieses biphasische Muster verloren. — 3. In Fettgewebe, welches mit dem beta-Blocker Kö 592 vorbehandelt wurde, wirkt Tolbutamid zusätzlich antilipolytisch; in Fettgewebe, welches mit dem alpha-Blocker Phentolamin vorbehandelt wurde, tritt dieser Effekt nicht auf. — Diese Ergebnisse führen zu dem Schluß, daß Tolbutamid wie Phentolamin zumindest im Konzentrationsbereich von 12,5–50 µg/ml auf eine Reaktion nach der Bildung von cyclischem 3',5'-AMP wirkt.

Key words: Tolbutamide, antilipolytic activity, lipolytic system, adipose tissue, adrenoceptive structures.

Besides the well-known beta cytotropic effect of sulfonylureas some other effects not mediated by insulin have been described (Feldmann and Lebovitz, 1969). Generally, the latter are summarized as "extra-pancreatic effects". Thus, sulfonylureas exert an inhibiting effect upon the lipolytic system of adipose tissue, as demonstrated by Renold *et al.* (1959), Stone *et al.* (1966), Stone and Brown (1967), Faulhaber *et al.* (1969) and Stork *et al.* (1969).

It is well established that lipolysis in rat adipose tissue is controlled by the tissue level of cyclic AMP (Butcher *et al.*, 1968; Hynie *et al.*, 1966), which is regulated by the adenyl cyclase system and phosphodiesterase activity. Agents stimulating either the activity of adenyl cyclase or inhibiting the activity of phosphodiesterase are known to increase the tissue

level of cyclic AMP and thus activate the triglyceride-splitting lipase system.

For further elucidation of the antilipolytic action of tolbutamide we have studied its effect upon norepinephrine-, dibutyl 3', 5'-AMP and theophylline-stimulated lipolysis. In addition, a possible interaction of tolbutamide with the alpha and beta adrenergic receptor sites in adipose tissue was investigated.

Method

Male Wistar rats of 120 to 160 g were fed ad libitum and sacrificed by decapitation. The epididymal fat pads were removed and washed in Krebs-Ringer-Bicarbonate-Albumin buffer (KRBA). 0.5 g adipose tissue was

Fig. 1. Tolbutamide action upon lipolysis stimulated with different agents in rat epididymal fat pieces. Glycerol, liberated by the stimulatory agents in the absence of tolbutamide served as control and was set as 100%. Depression of enhancement of lipolysis caused by tolbutamide was calculated as percent of control

cut into pieces and transferred to 25 ml polyethylene vials containing glucose-free KRBA buffer (2% albumin; pH 7.4). After addition of the various lipolytic agents and tolbutamide in different concentrations, the preparation was diluted to 10 ml. The vials were gassed with carbogen (95% O₂; 5% CO₂), closed tightly and incubated at 37°C in a Warburg metabolic shaker for 60 min. In experiments with *sympathicolytica*, phentolamine and Kö 592 were added 15 min before this incubation phase. At the end of the incubation period an aliquot of the medium was removed for determination of free glycerol (Eggstein and Kreutz, 1966), as an index of lipolytic activity (Wieland and Suyter, 1957). After correction for basal lipolysis, the glycerol release induced by the lipolysis-stimulating agents was expressed as 100% and the depression of glycerol release by tolbutamide and the sympatholytica was calculated.

Reagents, added *in vitro*:

1. Bovine serum albumin, "reinst", Behringwerke Marburg
2. Norepinephrine, Arterenol®, Farbwerke Hoechst, Frankfurt
3. Tolbutamide, Rastinon®, Farbwerke Hoechst, Frankfurt
4. Kö 592, Boehringer, Ingelheim
5. Theophylline, Euphyllin®, BYK Gulden, Konstanz
6. N⁶,2'-O-Dibutyl-adenosin-3',5'-monophosphat, cyclisch, Boehringer, Mannheim
7. Phentolamine, Regitin®, CIBA AG, Wehr/Baden

Results

In preliminary experiments, the dose-relationship of norepinephrine and theophylline on lipolysis was established. Both agents, but in different concentra-

Fig. 2. Dose response curves demonstrating the inhibitory activity of the alpha-blocker phentolamine and the beta-blocker Kö 592 upon norepinephrine-induced lipolysis

tions, exert nearly the same maximum lipolytic activity, a four-fold increase of basal glycerol release. In all further experiments, almost equipotent concentrations, causing a submaximal increase of lipolysis, i.e. 3×10^{-7} M norepinephrine and 1.25×10^{-3} M theophylline, were used. For dibutyryl 3', 5'-AMP we did not establish a special dose response curve. Based on the results of Aulich *et al.*, 1967, and Moskowitz and Fain, 1969, we have chosen a concentration of 10^{-3} M, which constantly enhanced the lipolysis, increasing glycerol release to 2.5-fold above basal values (1.09 ± 0.04 to 2.40 ± 0.17 mg%).

Tolbutamide caused a significant antilipolysis in low (12.5–25 μ g/ml) and high concentrations (200–500 μ g/ml). This inhibitory effect seemed to be partially lost in the concentration range 50–100 μ g/ml. The change from minor to strong inhibition of lipolysis was variable in concentrations from 50–200 μ g/ml, with the different lipolytic agents norepinephrine and theophylline. In contrast, using the synthetic nucleotide dibutyryl 3', 5'-AMP as stimulator of lipolysis, this biphasic pattern was lost, but the low concentrations of tolbutamide exerted an even stronger antilipolytic effect. The depression of glycerol release by high concentrations of tolbutamide was no longer demonstrable (Fig. 1).

Since epinephrine, norepinephrine etc. act via the adrenergic receptors *in vivo* and *in vitro* as potent stimulators of lipolysis, the alpha and beta sympathicolytic agents block lipolysis in a dose-dependent way (Fig. 2). Both substances are reported to exert their effect on different structures of the fat cell, the beta blocker Kö 592 on the adenylyl cyclase (Stock and Westermann, 1966) and the alpha blocker phentolamine on the triglyceride lipase system (Hynie *et al.*, 1969). Therefore, experiments with the combined administration of tolbutamide and the alpha or beta-blocking agents were carried out (Fig. 3). The sympathicolytic drugs were used in a concentration which, in preliminary experiments (Fig. 2), was found to cause a depression of lipolysis of about 30 % ($2.2 \cdot 10^{-7}$ M for Kö 592 and $7.5 \cdot 10^{-4}$ M for phentolamine; black bar in Fig. 3). Additional antilipolysis of tolbutamide was only observed in fat minces pretreated with Kö 592 (— — —), whereas the sulfonylurea failed to show any further effect in the presence of phentolamine (—). It can be stated also that in these experiments with Kö 592 the biphasic pattern to tolbutamide action remains visible.

Discussion

An antilipolytic effect of tolbutamide has already been described by several authors (Renold *et al.*, 1959; Stone *et al.*, 1966; Stone and Brown, 1967; Faulhaber *et al.*, 1969; Stork *et al.*, 1969), but in all these experiments, tolbutamide concentrations below 50 μ g/ml have not been studied. In this range of concentration

(12.5–50 μ g/ml), we were able to detect a considerable antilipolytic effect of tolbutamide, as already described elsewhere (Rosak *et al.*, 1970). Our results confirm the antilipolytic potency of tolbutamide *in vitro*. On the other hand, they do not support a simple dose-response relationship, but are in favour of a more complex situation, tolbutamide exhibiting its antilipolytic activity in two separate ranges of concentration. This biphasic pattern is observed if the stimulation of lipolysis is mediated by a direct or indirect accumulation of cyclic 3', 5'-AMP. In the presence of the synthetic nucleotide itself, the antilipolytic activity of tolbutamide is demonstrable only in low concentrations, but is missing in concentrations above 50 μ g/ml.

Fig. 3. Effect of tolbutamide on glycerol release of lipolysis stimulated with norepinephrine in epididymal rat adipose tissue pretreated with phentolamine — and Kö 592 ----. The black bar represents the inhibition of lipolysis caused by the blocking agents

This phenomenon prompts the suggestion that high concentrations of tolbutamide exhibit their antilipolytic activity by reactions which cause a depletion of the intracellular cyclic 3', 5'-AMP level. On the other hand, low sulfonylurea concentrations seem to interfere with a subsequent reaction, possibly with the activation of triglyceride lipase by the nucleotide itself.

Adipose tissue is known to behave biochemically and functionally as an organ innervated by the sympathetic (Stock and Westermann, 1966). With regard to these relations, the interference of tolbutamide with the adrenoceptive structures in adipose tissue has been investigated. The antilipolytic reaction of alpha and beta sympathicolytic agents on norepinephrine-stimulated lipolysis was taken as a model to be compared with the action of tolbutamide. In the concentration

range from 12.5–500 $\mu\text{g/ml}$ (4.6×10^{-5} to $1.85 \times 10^{-3}\text{M}$) tolbutamide is able to induce additional antilipolysis in adipose tissue pretreated with the beta blocker, whereas it is unable to do this in a tissue pretreated with the alpha blocker. Since we know nothing about the affinity and the intrinsic activity of tolbutamide with respect to the adrenergic receptors in adipose tissue, it is difficult to explain this phenomenon. Theoretically, one would expect an additive effect of two synchronously administered and synergistic agents, if they operate on different receptors. Thus, if two agents having affinity, but no intrinsic activity, compete for the same receptor, the substance with the lower affinity to the binding sites of the receptor would not be able to reveal its blocking properties. These theoretical considerations applied to our experiments would permit the following interpretations: The results obtained with the Kö 592-pretreated tissue demonstrate that tolbutamide does not operate upon the beta adrenergic receptor, but provides additional antilipolysis by blocking the alpha receptor. On the other hand, the inability of tolbutamide to increase the inhibition of lipolysis in the presence of phentolamine has to be explained by its lower affinity to the alpha receptor compared with that of phentolamine.

The biochemical site of action of phentolamine was shown by Peterson *et al.* (1968) to interfere with the reaction subsequent to the formation of cyclic 3', 5'-AMP. Furthermore, Hynie *et al.* (1969) were able to inhibit directly the triglyceride lipase activity in adipose tissue homogenates with the same substance.

Therefore, if tolbutamide acts in a similar way to phentolamine, it would be expected to interfere with the reaction subsequent to the formation of cyclic 3', 5'-AMP. In fact, tolbutamide exerted its antilipolytic activity in those experiments where dibutyryl 3', 5'-AMP was used as the lipolysis-stimulating agent. Further experiments should clarify whether this effect of the sulfonylurea is due to a direct inhibition of triglyceride lipase activation by cyclic 3', 5'-AMP.

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