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Effect of Imidazole on Glucose Tolerance and cAMP (39845)

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Introduction. The concentration of cAMP in any tissue reflects a balance between its formation from ATP and its conversion to 5'AMP. Phosphodiesterase (PDE), by virtue of its control of the rate of the cAMP breakdown, is one of the key enzymes in maintaining this balance.

Penhos *et al.* (1) studied the effect of aminophylline, a PDE inhibitor, on glucose tolerance in rats. They observed significant impairment of glucose tolerance with elevated plasma glucose and insulin levels. They postulated that aminophylline has a "diabetogenic" action resulting from a block in peripheral uptake of glucose despite increased insulin availability. If this blockade of glucose uptake were due to an inhibition of PDE, then imidazole, a stimulator of PDE (2), might be expected to facilitate the uptake of glucose into the peripheral tissues.

Materials and methods. Male, Sprague-Dawley rats, weighing 180-250 g, were fasted for 18-24 hr prior to use. They were anesthetized by intraperitoneal (ip) injections of sodium amytal (4 mg/100 g body wt) prior to glucose loading or administration of the drugs or saline.

At specified time intervals after ip or iv imidazole (20 mg/100 g body wt) or saline injection, groups of 10 rats were sacrificed and blood was drawn from the abdominal aorta for analysis. For the oral glucose tolerance test (OGTT), anesthetized rats were given a 50% glucose solution, 1 ml/100 g body weight, via gastric intubation followed immediately by an ip injection of 20 mg/100 g body weight of imidazole in 0.3 ml of saline or an equal volume of saline. For the iv glucose tolerance test (IVGTT) 50% glucose (0.5 ml/100 g body wt) was injected into the external jugular vein prior to imidazole or saline treatment. The external jugular vein was also used for iv injection of imidazole.

Glucose determinations were made with the glucostat reagent (3). Insulin was measured by the double-antibody method of Morgan and Lazarow (4), and free fatty acid (FFA) levels were determined colorimetrically (5).

For cAMP studies, 18 rats were randomly divided into three groups of 6 each. After anesthetization one group received imidazole (20 mg/100 g body wt), another received aminophylline (10 mg/100 g body wt), and the third received saline (0.2 ml/100 g body wt). Each rat was used for two time-interval measurements and each sample was carried out in duplicate. Samples of liver, abdominal muscle (rectus abdominis), and epididymal fat were excised and prepared for cAMP assay (6).

For the insulin tolerance test, insulin (0.1 unit/100 g body wt) and imidazole (20 mg/kg) or insulin and saline (control group) were injected ip in fasted, anesthetized male rats. Blood was drawn from the tail for glucose analysis. Student's t test was used for statistical analysis (7).

Results. Neither ip nor iv injection of imidazole significantly altered the blood glucose or insulin levels when compared to saline injection controls.

Figures 1 and 2 show the blood glucose and the plasma insulin levels, respectively, during the OGTT. Both glucose (30, 60, 120, and 180 min) and insulin (30 and 60 min) levels were significantly ($P < 0.001$) lower in the imidazole-treated rats. The characteristic changes in plasma FFA during the OGTT were similar in imidazole- and saline-treated animals (0.80 ± 0.05 μEq/ml at 0 hr, 0.43 ± 0.04 μEq/ml at 1 hr, and 0.69 ± 0.05 μEq/ml at 3 hr). The decreas-

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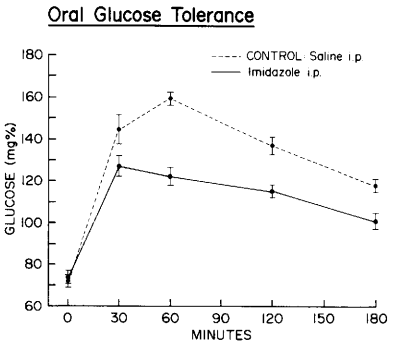

FIG. 1. The effect of ip imidazole (20 mg/100 g body wt) on blood glucose level during an OGTT (glucose 50%, 1 ml/100 g body wt). Each value is the average of 10 measurements with its SE.

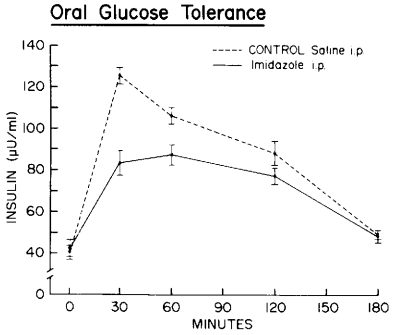

FIG. 2. The effect of ip imidazole (20 mg/100 g body wt) on plasma insulin level during an OGTT. Each value represents the average of 10 measurements with its SE.

ing levels of blood glucose during an insulin tolerance test (from 79 ± 1.5 mg% at 0 hr to 30 ± 1.9 mg% after 2 hr) were also unchanged by imidazole treatment. During an IVGTT, the effect of imidazole on glucose tolerance and insulin levels, which had been observed during the OGTT, was no longer in evidence. Also, imidazole had no significant effect on FFA levels during the IVGTT when compared to saline.

In another study, cAMP levels were determined at 30, 60, 120, and 180 min after treatment with either saline, imidazole, or aminophylline. It was observed that, 30 min after treatment with aminophylline, the cAMP concentrations in liver (1.10 ± 0.19 p*M*/mg tissue), muscle (1.10 ± 0.14 p*M*/mg tissue), and epididymal fat (0.4 ± 0.04 p*M*/mg tissue) were significantly ($P < 0.01$) higher than control values (0.64 ± 0.10 p*M*/mg liver, 0.45 ± 0.06 p*M*/mg muscle, and 0.22 ± 0.03 p*M*/mg adipose tissue). Imidazole treatment, however, did not affect cAMP levels significantly. Similarly, we observed a significant ($P < 0.01$) increase in plasma FFA concentration at 30 min (from 0.79 ± 0.01 to 1.0 ± 0.02 μEq/ml) following an ip injection of aminophylline, which correlated with the rise in cAMP during the same time interval. Again, imidazole treatment was without effect on the FFA levels.

Discussion. Turtle *et al.* (8) reported that aminophylline administration to rats resulted in a significant increase in the plasma glucose and insulin levels. Subsequently, Penhos *et al.* (1) showed that, during the OGTT and IVGTT, 10 mg/100 mg body weight of aminophylline produced an exaggerated rise in plasma glucose and insulin concentrations. If these effects were due to the inhibitory action of aminophylline on PDE, the opposite results might be expected with imidazole. In our studies, however, we found that imidazole had no effect on plasma insulin, glucose, or FFA during an IVGTT. Injection of 20 mg/100 g body weight of imidazole during the OGTT resulted in lower plasma glucose (Fig. 1) and insulin (Fig. 2), but not FFA.

These data, plus the fact that imidazole failed to lower the glucose levels beyond that which could be achieved by a single injection of insulin, suggest that the effect of imidazole on plasma insulin during an OGTT is not mediated via a direct effect on cAMP activity and insulin release. Another possible explanation is that imidazole leads to decreased glucose absorption from the gut and secondarily to a diminution in insulin release. This hypothesis is supported by the absence of an imidazole effect on either plasma insulin, glucose, or FFA when the drug is administered during an IVGTT.

Since the intracellular levels of cAMP have been shown to be an important controlling factor in many cellular processes, including insulin release from the β cells of the pancreas (9–11), it is likely that aminophylline's action might be mediated through a rise in cAMP level. There is no definitive proof, however, that aminophylline acts *in vivo* to inhibit PDE.

The inability of imidazole to alter significantly the IVGTT led us to investigate the

effect of this drug on cAMP *in vivo*. We found that administration of imidazole had no effect on cAMP in liver, muscle, or adipose tissue, while aminophylline increased cAMP in these tissues. The increase in insulin levels following aminophylline administration is due in part to the elevation of cAMP in the β cells (6). Since administration of aminophylline produces a block of glucose uptake by peripheral tissues (1), the resulting elevation of blood glucose would lead to further stimulation of insulin secretion. Aminophylline also exerts a lipolytic effect on tissue which is manifested by increased plasma FFA levels. This rise in FFA is paralleled by a simultaneous increase of cAMP in epididymal fat. Imidazole, on the other hand, was not observed to change cAMP levels *in vivo*, which suggests either that cAMP levels *in vivo* are subject to compensatory control mechanisms (feedback) which override an *in vivo* imidazole effect, or that imidazole does not stimulate PDE *in vivo* as it does *in vitro*. Cheung (12, 13) reported that both ATP and inorganic pyrophosphate (PPi), the substrate and product, respectively, of adenylate cyclase, are effective inhibitors of PDE. Thus, it is suggested that these inhibitors of PDE may prevent imidazole from altering cAMP levels *in vivo*. Imidazole, under these conditions, would be ineffective as a regulator of cAMP levels or of plasma substrate concentrations.

Summary. The *in vivo* effects of a phosphodiesterase (PDE) stimulator, imidazole, and a PDE inhibitor, aminophylline, were studied in rats. Imidazole caused an impairment of OGTT but not IVGTT. Aminophylline caused a rise in cAMP levels of liver, muscle, and adipose tissue, whereas imidazole did not alter the cAMP levels in these tissues. It is concluded that the *in vitro* effect of imidazole is not manifest *in vivo* and the differing effects of the drug observed during the two routes of glucose administration can be explained better by interference with absorption of glucose through the GI tract rather than alteration of tissue cAMP levels.

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