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Effects of adrenergic receptor blockers on adrenaline and nicotine-induced hyperglycaemia in the rat

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Summary

Studies in fasted, anaesthetized rats have shown that adrenaline and nicotine-induced hyperglycaemia is mediated via activation of β-adrenoceptors. The hyperglycaemic response to nicotine is different from that of adrenaline in that, unlike adrenaline hyperglycaemia, nicotine hyperglycaemia is characterized by a long latent period, delayed attainment of peak of response and sustained hyperglycaemia throughout the 120 minutes post-injection observation. Comparison with previous studies in dogs showed that different receptors are involved in nicotine hyperglycaemia in fasted dogs and fasted rats.

Résumé

Les études des rats anesthésiás et en jeûne ont montré que l'hyperglycémie induite par l'adrénaline et la nicotine est mediatée à travers l'activation du β-adrenocepteur. La réponse hyperglycémique à la nicotine est différente de celle de l'adrenaline en ce sens qu' à la différence de l'hyperglycémie induite par l'adrenaline, l'hyperglycémie induite par la nicotine se caractérise par une longue période latente, d'une réponse d'apogée retardée et d'une hyperglycémia soutenue durant toute la durée de 120 minutes que l'observation post-piqure a durée. Une comparaison avec des études préalables avec les chiens a montré que des récepteurs différênts se sont manifestés dans l'hyperglycémie induite par la nicotine avec les chiens qui jeûnent et avec les rats qui jeûnent.

Introduction

Adrenaline and nicotine are well-known hyperglycaemic agents. While adrenaline acts directly by stimulating release of glucose from glycogen stores in the muscles and liver, nicotine acts indirectly by first causing adrenaline release from the adrenal glands and the adrenaline thus released causes hyperglycaemia[1,2].

The hyperglycaemic response to adrenaline and other sympathomimetic amines has been extensively studied; nevertheless, the question of the receptor mediating the response has generated considerable controversy[3]. The response is highly complex and is incompletely understood. For example, β-adrenoceptor drugs have been reported as capable of abolishing[4,5]; reducing[6,7,8]; or having no effect whatever[9,10,11,12]; on adrenaline hyperglycaemia. On the other hand, some workers found that α-adrenoceptor blocking drugs may totally block[13], reduce[8,14,15] or have no effect[5,16] on adrenaline hyperglycaemia.

The situation is further compounded by the considerable species variation that have been reported in the metabolic responses to adrenergic agents and their antagonists[17].

Also, whether the animal is fed or fasted affects the adrenoceptors involved in adrenaline hyperglycaemia. Thus, hyperglycaemic response in fed rat is mediated by α-receptors and in the fasted rat, the response is mainly due to the activation of β-receptors[18,19]. Indeed, some workers were only able to prevent adrenaline hyperglycaemia with a combination of alpha and beta adrenoceptor blockers[5,8,20].

Unlike adrenaline and other sympathomimetic amines, the receptors involved in nicotine-induced hyperglycaemia have not received much attention from research workers. The only information available to us after an extensive search of the literature is from a study in dogs by Grayson and Oyebola[21]. They found that β-blockade with propranolol significantly reduced the hyperglycaemic

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response to nicotine infusion while alpha-blockade with prazosin abolished the large increases in arterial and venous glucose levels caused by nicotine in untreated dogs. This led to the conclusion that the effect of nicotine on blood glucose in the dog is predominantly α-mediated. There has been no report on the receptors involved in nicotine-induced hyperglycaemia in other animal species.

The above conflicting reports on the nature of receptors mediating adrenaline hyperglycaemia seem to us sufficient to warrant further investigation of the adrenoceptors mediating adrenaline-induced hyperglycaemia. Also, apart from the dearth of information on the receptors involved in nicotine-induced hyperglycaemia, in view of the extensive exposure of man to nicotine through smoking and in Nigeria, through medication with cow's urine concotion in the treatment of convulsions[22], this study also invested the role, if any, of adrenoceptors in nicotine-induced hyperglycaemia. The latter part of this study was informed by the reports that nicotine causes hyperglycaemia via the release of adrenaline[1,2].

Materials and methods

Male albino rats of the Wistar strain weighing 200-260 gm were used for the experiments. Each animal was fasted for 18-24 hours before the start of an experiment. Anaesthesia was induced with 0.6 ml/100gm of 25% (w/v) urethane solution administered intraperitoneally. The preparation of the animals and blood sampling for glucose estimation were as previously described[23].

Adrenaline experiments

The adrenaline experiments were carried out on three groups of rats (Groups I, II and III) consisting of 8 rats per group. Rats in group I were given a bolus intravenous injection of adrenaline 50 μg/kg each. Group II rats were pretreated with prazosin 0.2μg/kg followed by a bolus intravenous injection of adrenaline, 50 μg/kg. Group III rats were pretreated with propranolol 0.5 mg/kg followed by adrenaline, 50 μg/kg injection. In order to ascertain the subtype of β-receptors involved in the changes observed in the blood glucose levels in the propranolol treated rats, two more groups of rats were pretreated with either 8 μg/kg IPS 339 (β_2-blocker) or 0.2 mg/kg practolol (β_1-blocker) and the adrenaline, 50 μg/kg, injection was repeated. In each of the experiments, a resting blood sample was taken before the adrenaline injection. After the injection, blood samples for glucose estimation were obtained at 1 min, 2 min, 5 min, 30 min, 45 min, 60 min and 90 min post-injection. All animals pre-treated with alpha or beta blocker were allowed 30 minutes post-treatment for the drugs to take effect and a blood sample was taken for glucose estimation before they were challenged with adrenaline (or nicotine for the nicotine experiments).

Nicotine experiments

The experiments were repeated using nicotine in place of adrenaline. Three groups of rats consisting of 8 rats per group, were also used. The control group was given nicotine, 50 μg/kg as a bolus i.v. injection. Groups II and III were pretreated with prazosin (0.2 mg/kg) and propranolol (0.5mg/kg) respectively before they were given bolus injection of 50 μg/kg nicotine. Blood damples were taken before and after nicotine injection in each group and sampling was continued for 120 minutes post-injection.

0.9% Saline injections

The experiment was repeated in a group of 8 rats given 0.9% saline i.v. in the same volume as the adrenaline or nicotine injections.

Estimation of blood glucose

Each sample of blood (0.05 ml) was immediately transferred into 2.95 ml of protein precipitant. Blood glucose concentration was measured using the glucose oxidase method of Trinder[24].

A *t*-test of difference between two independent sample means[25] or a paired *t*-test was used as appropriate as in a recent study[23]. *P* values of 0.05 or less were taken as statistically significant.

Results

The results are shown in Figures 1, 2, 3 and 4.

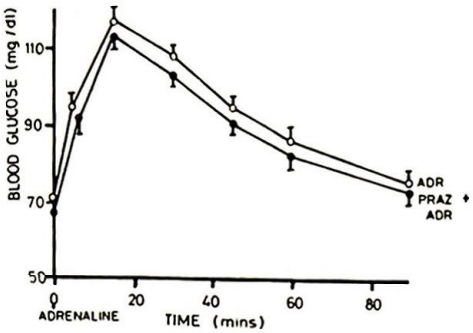

Fig. 1: The effects of adrenaline (50 µg/kg i.v.) on blood glucose levels in untreated (0-0) and prazosin treated (0.2 mg/kg) (⊕ — ⊕) rats. Note the marked hyperglycaemia which reached a peak 15 min post-injection and the non-significant reduction of adrenaline hyperglycaemia by prazosin. The points are Mean ± S.E. of 8 determinations.

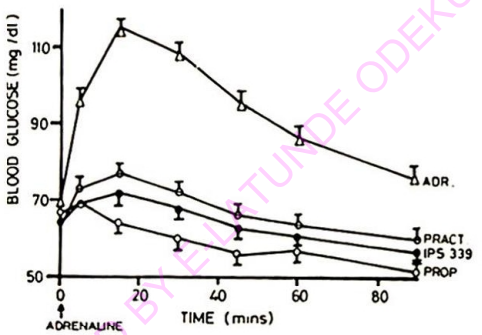

Fig. 2: The effect of adrenaline (50 µg/kg i.v.) on blood glucose levels in untreated (Δ — Δ) and in rats treated with the beta-blockers practolol, 0.2 mg/kg, (θ — θ), IPS 339, 8 µg/kg (• — •) and propranolol, 0.5 mg/kg (O — O). Note the abolition of adrenaline hyperglycaemia by propranolol and its marked reduction by practolol and IPS 339. The points are Mean ± S.E. of 8 determinations.

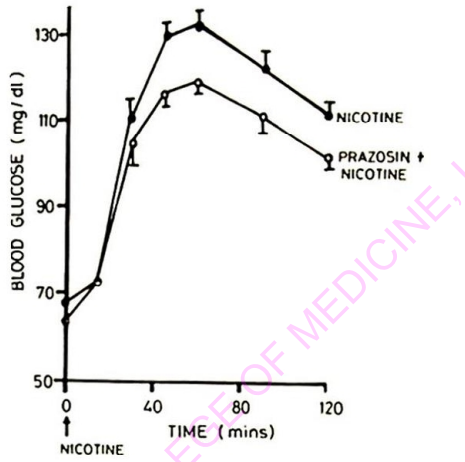

Fig. 3: The effects of nicotine (50 µg/kg i.v.) on blood glucose levels is untreated (• — •) and prazosin treated, 0.2 mg/kg (O — O) rats. Note the long latent period of about 15 min before the hyperglycaemic response to nicotine and the sustained hyperglycaemia, even at 120 min post-injection. Prazosin caused a significant reduction ($P < 0.05$) in nicotine hyperglycaemia from 45 to 120 min post- injection. The points are Mean ± S.E. of 8 determinations.

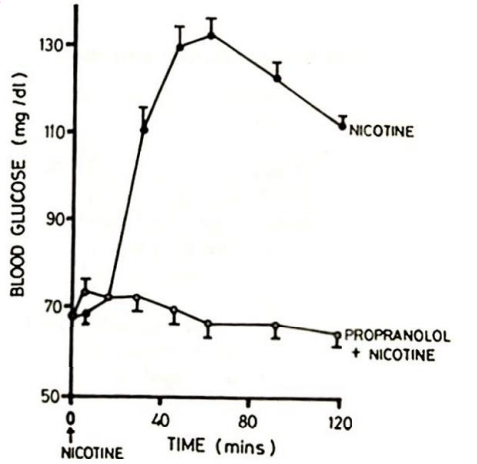

Fig. 4: The effects of nicotine (50 µg/kg i.v.) on blood glucose levels is untreated (• — •) and propranolol-treated, 0.5mg/kg (O — O) rats. It should be noted that propranolol abolished nicotine hyperglycaemia. The points are Mean ± S.E. of 8 determinants.

Effects of α-blocker on the response to adrenaline

Figure 1 shows the effect of adrenaline injection on blood glucose levels in untreated and prazosin-treated rats. Adrenaline caused a significant increase in blood glucose level ($P < 0.001$). This increase was obvious within the first minute of injection. The glucose increase attained a peak at 15 minutes post-injection, thereafter, it declined gradually for the remaining post-injection observation period. Prazosin had no significant effect on adrenaline-induced hyperglycaemia. It should be noted that prazosin alone did not cause a significant change in blood glucose level even at 30 minutes after it was injected.

Effect of β-blockers on the response to adrenaline

Figure 2 shows the effects of beta-adrenergic blockers. Pre-treatment of the rats with propranolol completely abolished the hyperglycaemic response to adrenaline. Also, practolol and IPS 339 significantly reduced the hyperglycaemic response to adrenaline ($P < 0.001$). Again, it is important to note that none of the three beta-blockers used produced a significant change in blood glucose level even 30 minutes after each was injected.

Effects of α-blocker on the response to nicotine

Figure 3 shows the effect of nicotine on blood glucose levels in untreated and prazosin-treated rats. In the untreated group, the hyperglycaemic response became evident 15 minutes post-injection. Blood sampling at 1 min, 2 min, and 5 min in the preliminary experiments did not reveal any change in blood glucose level. Therefore, the 1 min, 2 min and 5 min samples were discontinued in subsequent experiments. The blood glucose level reached a peak at 60 minutes post-injection and remained markedly elevated throughout the post-injection observation period. Although prazosin caused a significant reduction of hyperglycaemia produced by nicotine as from 45 min post-injection to the end of the observation period, blood glucose in the prazosin-treated rats non-the-less remained markedly elevated throughout the post-injection period.

Effect of β-blocker on the response to nicotine

Figure 4 shows that propranolol abolished the nicotine-induced rise in blood glucose level. The effect is highly significant ($P < 0.001$).

When the glycaemic response to adrenaline and nicotine are compared (Figures 1 and 3), three main differences emerge. These are the delayed onset, delayed peak and sustained hyperglycaemia of the nicotine response compared with the adrenaline response.

Effect of 0.9% saline on blood glucose

Equal volume of 0.9% saline as the adrenaline or nicotine injection had no effect on blood glucose.

Discussion

The rise in blood glucose following adrenaline or nicotine injection in this study is consistent with their known pharmacological effect on blood glucose. Since 0.9% saline injection had no effect on blood glucose, the hyperglycaemic effect of adrenaline and nicotine could not be ascribed to the stress of the injections.

The non-significant reduction of adrenaline-induced hyperglycaemia by prazosin suggests that the increase in blood glucose level produced by adrenaline is not mediated by alpha receptors. This finding is consistent with earlier reports in rabbits[26] and dogs[13].

The complete abolition of adrenaline-induced hyperglycaemia by propranolol agrees with previous reports in the dog[13], rat[19], cat[27] and man[28]. Since the β-receptors are present mostly in the skeletal muscles[28,30,31,32] and the liver glycogen store is just 1% of normal in fasted rats[19,27,28], most of the glucose released into the blood after administration of adrenaline is most probably produced from lactate from the skeletal muscle through the Cori cycle[33].

The observed blockade of adrenaline-induced hyperglycaemia by IPS 339 (a β_2-Blocker) is consistent with the view that muscle glycogenolysis is β_2-mediated[31]. However, the present study also showed the involvement of β_1-receptors since practolol almost totally abolished adrenaline hyperglycaemia. We are led by this observation to conclude that muscle glycogenolysis in the rat is mediated by both β_2 and β_1-receptors and not just by β_2-receptors as claimed earlier by Arnold and Selbens[31].

Nicotine and blood glucose

The present study, as far as we are aware, is the first study of the adrenoceptors involved in nicotine

hyperglycaemia in the rat. Previous studies in dogs[1] and cats[2] showed that the hyperglycaemia induced by nicotine is mainly due to the adrenaline released from the adrenal medulla. The latter effect is mediated via the stimulation of acetycholine-nicotinic receptors[34,35,36]. It is interesting to note that while prazosin abolished nicotine-induced hyperglycaemia in the dog and propranolol only reduced it[21], the reverse occurred in the rat in the present study. This difference is most probably due to species variation. However, the abolition of nicotine hyperglycaemia by propranolol provides strong proof that nicotine is acting by causing adrenaline release.

There are two other observations from our nicotine experiments that are difficult to reconcile with the findings of Tsuijimoto *et al*[1] and Grayson and Oyebola[21]. The first is that unlike the rapid response reported by the latter authors in the dog, there was a rather long latent period of over 10 minutes before the hyperglycaemic response to nicotine in the rat was observed. Secondly, nicotine-induced hyperglycaemia was sustained, unlike in the studies of Tsuijimoto *et al*[1] and Grayson and Oyebola[21] in which blood glucose returned to basal level at 15 minute post-injection. The reason for these differences is obscure. Further studies will be required to clarify this.

The present study however showed conclusively that adrenaline-and nicotine-induced hyperglycaemia in the rat are mediated via beta- adrenoceptors. The alpha-adrenoceptors play no significant role in this response. This conclusion is consistent with the findings of Hendler and Sherwin on adrenaline-induced hyperglycaemia in man[31]. This suggests that in spite of the several species variation reported in the receptors mediating adrenaline and nicotine hyperglycaemia, the rat and man most probably have identical receptors in this response.

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