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Effects of calcium channel blockers on nicotine-induced hyperglycemia in the rat

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Summary
Effects of nifedipine and verapamil on nicotine-induced hyperglycemia were studied in fasted, anesthetized male rats. Blood glucose was measured using the glucose oxidase method. Nifedipine (0.05-0.20 mg/kg i.p.) significantly reduced nicotine-induced hyperglycemia in a dose - dependent manner. While lower doses (0.1.0-1.0 mg/kg i.p) of verapamil did not effect nicotine-induced hyperglycemia, higher doses 95.0 - 10 mg/kg i.p) of verapamil caused significant reduction but did not abolish it.

Both nifedipine and verapamil had no significant effect on the basal blood glucose levels at any dose used. These results seem to suggest that the reduction in nicotine - induced hyperglycemia is related to inhibition of some of the processes by which nicotine induced hyperglycemia.

Keywords: *Verapamil, nifedipine, nicotine-induced hyperglycemia, rat.*

Résumé
Les effets de la nefedipine et de la Jeune verapamil sur l'hyperglycemie induite par la necotine avaient été etudiés chez les rats maile anestesies et a jeune. Le taux de glucose du sang avait été mesuré par la methode du glucose oxidase. La nefedipine significativement l'hyperlycernie induite par la nicontine, dependant de la dose. Alorsque, les faible doses de Verapainil (0, 1 a mg/kg ip) n'avauent pas affecte l'hyperglyceimee induite par la nicotine, les doses forte (5, 0 a 10mg/kg ip) de verapmil avaient causé une reduction significative vous n'avaient pas aneantis l'hypertension.
La nefedipine et la verapamil n'avaient aucune dose au significatif sur le taux basal de glucose. Les resultats semiblent suggeré que la reduction de l'hyperglycemie induite par la nicotine étu se fait par rapport a l'inhibition de certains procedes par lesquels la nicotine induit l'hyperglycemie.

Introduction

Nicotine has been widely studied especially as it relates to the smoking habit and carcinogenesis [1,2]. It is the principle alkaloid contained in tobacco and is usually implicated as the agent responsible for the acute biological effects of tobacco smoking [3]. The effect of nicotine on blood glucose has been extensively investigated [4,5,6,7]. Previous studies in dogs [5,6], cats [4] and rats [7] have shown that the hyperglycemia induced by nicotine is mainly due to the adrenaline released from the adrenal medulla. The later effect is mediated via the stimulation of acetylcholine - nicotinic receptors [8,9,10] and subsequently, activation of voltage-dependent calcium permeability of the cell membrane [10].

It is well acknowledged that calcium channel blockers inhibit calcium influx through the voltage-dependent calcium channels to reduce vascular tone, hormone secretion and nerve impulse transmission [11]. Several investigators have also demonstrated that calcium channel blockers such as verapamil and nifedipine are capable of inhibiting glucose - stimulated insulin release by pancreatic is lets *in vivo* [12,13,14]. Although some reports have been published concerning the effects of calcium channel blockers on glucose tolerance [15,16], the effects of these agents on glucose homeostasis are not clearly understood. Furthermore, a recent study [17] showed that verapamil and nifedipine potentiated adrenaline - induced hyperglycemia in rats. However, the effects of calcium channel blockers on hyperglycemia induced by other agents such as glucagon, growth hormone, thyroxine, cortisol and nicotine are largely unknown. Also, since nicotine mediates its hyperglycaemic effect through the release of adrenaline from the adrenal medulla, the question then arises; will calcium channel blockers also potentiate nicotine-induced hyperglycemia as was the case with adrenaline-induced hyperglycaemia?

The present study was therefore designed to investigate the effect of verapamil and nifedipine on nicotine-induced hyperglycemia.

Materials and methods

Male albino rats of the Wistar strain weighing 250-300g were used for the study. Each animal was fasted for 18-24h before the start of experiments. Anesthesia was induced with sodium pentobarbitone (45mg/kg i.p). The preparation of the animals and blood sampling for glucose estimation were as previously described [7].

Experimental Procedure

The initial experiments were carried out on three groups of rats (with 8 rats per groups). Rats in group I were given a bolus injection of nicotine, 50μg/kg as in a previous study [7]. Group II rats were pretreated with nifedipine (0.05, 0.10, 0.25 and 0.3mg/kg i.p) followed by i.v injection of nicotine, 50μg/kg. Rats in group III were pretreated with verapamil (0.1, 0.5, 1.0, 5.0, 10.0, 15.0 and 20.0mg/kg i.p) followed by a bolus i.v. injection of nicotine, 50μg/kg. In each of the experiments, a

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resting blood sample (0.05ml per sample) for glucose estimation was taken before nicotine injection was given. After the injection, blood samples for glucose estimation were obtained at 0 min, 1 min, 5min, 10 min, 30 min, 45 min, 60 min, and 90 min, post - injection. After every two samples of blood taken, the volume of blood withdrawn in the two samples was replaced with an i.v. injection of an equal volume (0.1ml) of 0.9% saline so as to maintain a stable blood volume. All the animals pretreated were allowed 30min post-treatment for the drug to take effect before nicotine was injected.

The experiment was then repeated in another group of 8 rats given 0.9% saline I.V in the same volume as the nicotine injections and they served as the control.

Each sample of blood (0.05ml) was immediately transferred into 2.95ml of protein precipitant. Plasma glucose was measured using the glucose oxidase method [18]. Statistical analysis of the results was performed using the student's t-test for unpaired data. Data were regarded as statistically significant at p values of 0.05 or less.

Results

The results are shown in figures 1 and 2. The values plotted in figures 1 and 2 were the means ± S.E.M. of the glucose levels over the period of observation.

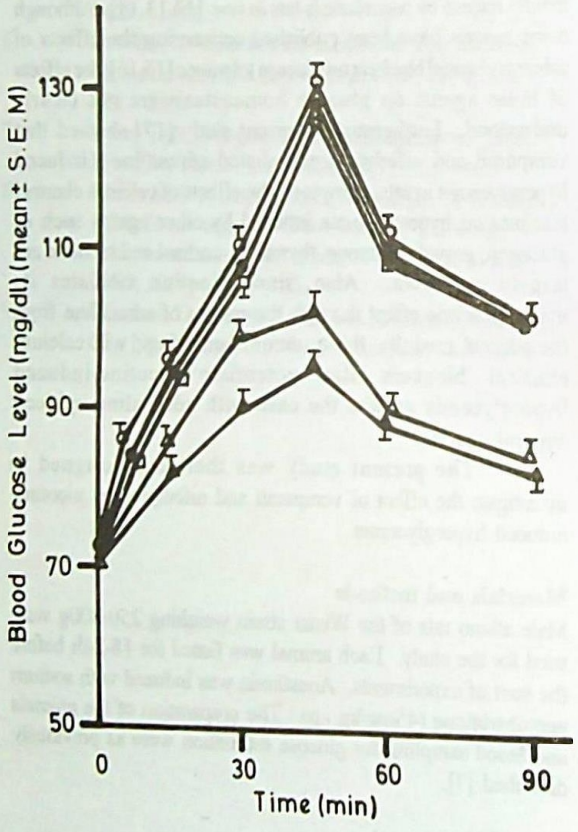

Figure 1
Effect of nicotine (50 µg/kg iv.) on blood glucose levels in untreated (0—0) and nifedipine (0.5 mg/kg (• — •), 0.10 mg/kg (Δ—Δ) and 0.2mg/kg (Δ—Δ) treated rats. Note that nifedipine did not abolish nicotine - induced hyperglycemia n = 8.

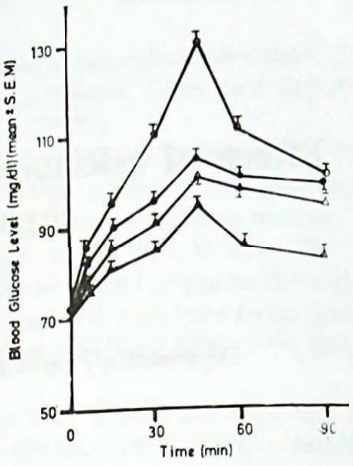

Figure 2
Effect of nicotine (50 µg/kg i.v) on blood glucose levels in untreated (0—0) and verapamil, 0.1 mg/kg (•—•), 1.0 mg/kg (□—□), 5.0 mg/kg (Δ—Δ) and 10.0 mg/kg (Δ—Δ) treated rats. Note that verapamil at higher doses only reduced and did not abolish nicotine-induced hyperglycemia n = 8.

Effect of nifedipine on response to nicotine

Figure 1 shows that intravenous injection of nicotine produced an increase in blood glucose which became evident after the first 15 min post-injection. The blood glucose reached a peak 45min post-injection and remained significantly higher than the basal values throughout the remaining post-injection observation period. When the animal was pretreated with nifedipline (0.05 - 0.5mg/kg i.p.), there was a significant reduction in the nicotine - induced hyperglycaemia in a dose-dependent manner (Figure 1). At a dose of 0.2mg/kg, the maximum effect of nifedipine on nicotine - induced hyperglycemia was produced. Higher doses of nifedipine did not cause further reduction in blood glucose level compared with the 0.2mg/kg dose. Nifedipine alone had no significant effect on the basal glucose level.

Effect of verapamil on the response to nicotine

The effect of verapamil on nicotine-induced hyperglycaemic is shown in Figure 2. Verapamil at lower doses (0.1-1.0mg/kg i.p) did not influence the nicotine-induced hyperglycemia throughout the time course of investigation. However, at higher does of verapamil (5.0, and 10.0mg/kg i.p) the nicotine-induced hyperglycemia was significantly reduced. Higher doses of verapamil did not cause further reduction of nicotine-induced hyperglycemia. Verapamil alone, had no significant effect on the basal blood glucose level.

Effect of 0.9% saline on blood glucose

Equal volume of 0.9% NaCl as the nicotine injections used in this study had no effect on blood glucose in all the rats in the control group.

Discussion

The increase in blood glucose produced by nicotine in this study is consistent with the findings of other workers [4,5,6,7]. The fact that higher doses of calcium channel blockers than those reported to have produced maximum blockade in this study did not produce further reduction in nicotine-induced hyperglycemia showed that calcium channel blockade with the doses used in this study was adequate. This is supported by

the works of Davis *et al.* [12] and Malaisse - Lange *et al.* [13] who achieved total calcium channel blockade with 0.2mg/kg of nifedipine and 8.0mg/kg of verapamil.

The mechanism by which calcium channel blockers reduced nicotine-induced hyperglycemia is not clear. However, it is unlikely that the reduction is mediated through stimulation of adrenal gland and release of adrenaline. This is because a recent study from our laboratory [17] showed that calcium channel blockers potentiated adrenaline-induced hyperglycemia. Therefore, the mechansim for the reduction of nicotine-induced hyperglycemia by nifedipine and verapamil may be largely related to inhibition of some of the processes involved in nicotine-induced hyperglycemia. For instance, Amy and Kirshner [19] have shown that nicotine-induced catecholamine secretion from the adrenal medulla is calcium dependent. The fact that calcium channel blockers had no effect on the basal blood glucose level also supports the possibility that the effect of calcium channel blockers on nicotine- induced hyperglycemia is via the process(es) by which nicotine induces hyperglycemia. Since nicotine-induced adrenaline release from the adrenal medulla is calcium-dependent [19], it seems reasonable to suggest that nifedipine and verapamil reduced nicotine-induced hyperglycaemia as shown in this study by interfering with the availability of adequate calcium needed for adrenaline release. Further studies, including measurement of plasma level of adrenaline in rats given nicotine, with and without calcium channel blockers pretreatment, will be required before final conclusions can be drawn on the mechanism of the observed effects of nifedipine and verapamil in this study.

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