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THE EFFECT OF SELECTIVE AND NON-SELECTIVE β-ADRENOCEPTOR BLOCKADE, AND OF NALOXONE INFUSION, ON THE HORMONAL MECHANISMS OF RECOVERY FROM INSULIN-INDUCED HYPOGLYCAEMIA IN MAN

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The rise in plasma adenosine-3',5'-monophosphate occurring in response to insulin induced hypoglycaemia in normal human subjects, was abolished by non-selective β-adrenoceptor blockade but unaffected by selective β_1-adrenoceptor blockade. This implies that the rise is secondary to β_2-adrenoceptor stimulation. The abolition of this rise by non-selective β-adrenoceptor blockade had no pronounced effect on the recovery from hypoglycaemia. Endogenous opiate receptor blockade with naloxone had no significant effect on the recovery from insulin induced hypoglycaemia, or the hormonal mechanisms involved.

Keywords selective, non-selective β-adrenoceptor blockade hormonal response adenosine cyclic monophosphate hypoglycaemia naloxone

Introduction

Hypoglycaemia is known to produce a rise in plasma adenosine-3',5'-monophosphate (cAMP) concentration which can be blocked by the prior administration of propranolol, a non-selective β-adrenergic receptor blocking agent (Hamet *et al.*, 1975). The rise in cAMP is hence thought to be secondary to the increase in activity of the sympathetic nervous system which occurs with hypoglycaemia. Whereas it has been suggested that selective β_1-adrenergic receptor blockade might be safer than non-selective β-adrenoceptor blockade in a patient prone to hypoglycaemia, the effect of selective β_1-adrenergic receptor blockade on the cAMP response to hypoglycaemia has not previously been investigated.

Opioids have been reported to stimulate central sympathetic outflow in rats (Van Loon *et al.*, 1981) and to stimulate the secretion of insulin and glucagon in man (Reid & Yen, 1981). It is therefore possible that an opiate antagonist would alter the response to hypoglycaemia and we have looked for evidence of this.

Methods

Five healthy subjects (two male and three female) aged 22–27 years were studied, using protocols that had been passed by the district ethics committee. All were within 10% of their ideal body weight. During the studies the subjects took their normal diet and usual exercise but fasted overnight before each experiment. Insulin tolerance tests were performed on each subject using four different experimental protocols given in a previously randomised order. Before three of the tests the subjects took either (a) propranolol 80 mg twice daily, (b) atenolol 100 mg once daily (and a placebo tablet at night) or (c) placebo in a double-blind regime using identical looking tablets. These were started two weeks prior to the test with the final dose taken 1 h before insulin was given. On the fourth occasion an infusion of naloxone, 10 mg, was started 10 min before insulin was given and continued until the end of the 2 h study.

Following their overnight fast the subjects rested on couches and after an initial blood sample an intravenous dose of unmodified soluble insulin (0.15 U/kg) was given. Blood was taken through an intravenous cannula kept patent with heparinised saline. Further blood samples were taken at 15, 30, 45, 60, 90 and 120 min post-insulin, the subject's pulse rate and blood pressure having previously been measured.

Glucagon was measured by radioimmunoassay (Alford *et al.*, 1977), cAMP was assayed by saturation analysis (Brown *et al.*, 1971) and noradrenaline and

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adrenaline by COMT radioenzymatic assay (Brown *et al.*, 1981). Blood glucose was measured by a glucose oxidase method using an auto-analyser. All measurements were made blind by the personnel performing the assays. Statistical analysis was assessed by Wilcoxon's rank sum test.

Results

Pulse rate and blood pressure

Subjects taking placebo showed an increase in their pulse rate during hypoglycaemia which returned to the resting level by the end of the test (Figure 1). The infusion of naloxone made no difference to the pulse rate. The subjects on atenolol had lower pulse rates than when on placebo but showed a similar rise in pulse rate during hypoglycaemia whereas the subjects on propranolol experienced a fall in pulse rate at this time. One subject on propranolol experienced extreme bradycardia with a pulse of 28 beats/min at the time of the nadir of blood glucose.

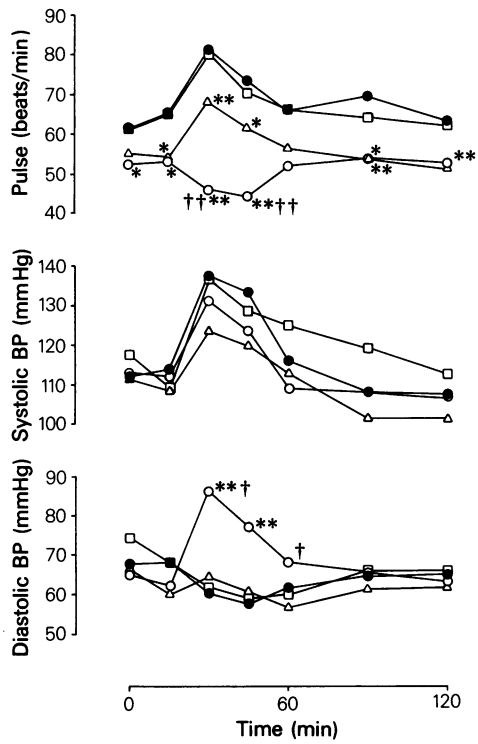

Figure 1 Mean values of pulse rate, systolic and diastolic blood pressures during insulin tolerance tests performed on subjects taking placebo (●), propranolol (○), atenolol (△) or having a naloxone infusion (□). (* $P < 0.05$, ** $P < 0.01$ when compared to placebo; † $P < 0.05$, †† $P < 0.01$ when compared to atenolol).

Changes in systolic and diastolic blood pressure are shown in Figure 1. β-adrenoceptor blockade made no significant difference to resting blood pressure. In the control study there was a rise in systolic and a fall in diastolic blood pressure during hypoglycaemia ($P < 0.05$). There was no significant change in this response in the naloxone or atenolol treated groups. Propranolol, however, resulted in a marked increase in diastolic pressure during hypoglycaemia ($P < 0.01$).

Plasma glucose

Within all subject groups a nadir in blood glucose occurred 30 min after the injection of insulin (Figure 2). At this stage the value for subjects on atenolol was just significantly lower than for subjects on placebo ($P = 0.05$) and on propranolol ($P < 0.05$) but at no other stage were significant changes found between placebo, propranolol, atenolol and naloxone. The rate of recovery of blood glucose from 45 to 120 min was slower with propranolol (0.58 ± 0.53 mmol l^{-1} h^{-1}) when compared to placebo (1.10 ± 0.47 mmol l^{-1} h^{-1}), however this did not achieve statistical significance. The recovery rate with atenolol showed no depression.

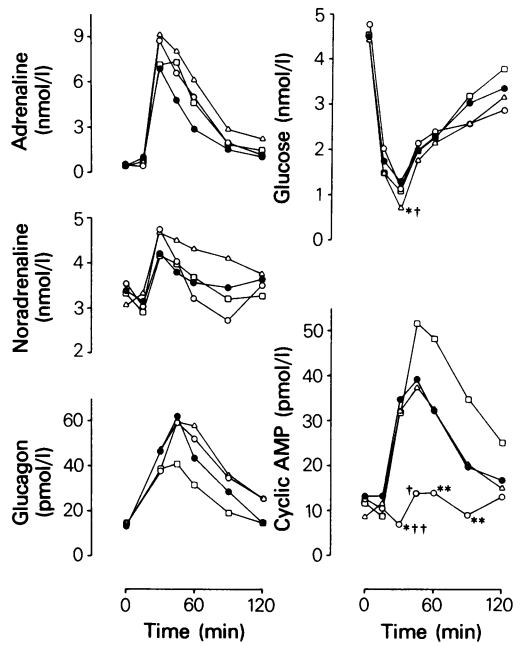

Figure 2 Mean values of glucose, cAMP, glucagon and catecholamine levels during insulin tolerance tests performed on subjects taking placebo (●), propranolol (○), atenolol (△) or having a naloxone infusion (□). (* $P < 0.05$, ** $P < 0.01$ when compared to placebo; † $P < 0.05$, †† $P < 0.01$ when compared to atenolol).

cAMP levels

There was no significant difference in the initial cAMP concentration in any subject group. After the injection of insulin the cAMP levels in the control group started to rise after 15 min reaching a peak at 45 min and virtually returned to the starting level by 120 min (Figure 2). The administration of atenolol or naloxone produced no significant change in the levels of cAMP from those on placebo, but the administration of propranolol totally abolished the plasma rise in cAMP.

Adrenaline and noradrenaline

Adrenaline levels rose 15 min after insulin to reach a value approximately 15 times the initial value (Figure 2). Noradrenaline levels rose over a similar time course but only by approximately 25% (Figure 2). Both adrenaline and noradrenaline reached peak values at 30 min and declined slowly thereafter. Both were unaffected by propranolol, atenolol or naloxone.

Glucagon

Following insulin plasma glucagon rose to four times its initial value at 45 min and declined to normal by 120 min (Figure 2). Neither propranolol, atenolol or naloxone caused a significant change in this response.

Discussion

Our cardiovascular findings corroborate those of previous studies (Davidson *et al.*, 1977; Lager *et al.*, 1979; Lloyd-Mostyn & Oram, 1975). The decreased pulse rates, during hypoglycaemia, whilst the subjects were taking propranolol or atenolol, suggests that their β-adrenoceptors were well blocked at the time of study. Of particular importance is the marked rise in diastolic blood pressure that occurred with hypoglycaemia when the subjects were taking propranolol. This is presumably due to blockade of β_2-vasodilatory receptors allowing an unopposed stimulation of α-adrenergic receptors, causing vasoconstriction, by both circulating catecholamines and the sympathetic nervous system. The concomitant bradycardia is presumably reflex in origin as it is abolished by atropine (Lloyd-Mostyn & Oram, 1975). This rise in diastolic blood pressure might be clinically significant in hypertensive patients, who are prone to attacks of hypoglycaemia. Indeed a case of severe hypertension associated with hypoglycaemia in a hypertensive woman taking propranolol has been reported (McMurtry, 1974). A β_1-selective adrenoceptor blocker would seem to be a safer alternative.

Whether β-adrenergic receptor blockade impairs the recovery from hypoglycaemia has been much debated and the evidence has been reviewed by Cryer (1981). In general the evidence suggests that β-adrenergic receptor blockade has no effect on the glucose nadir following intravenous insulin, but that glucose recovery might be impaired if the hypoglycaemia is sufficiently pronounced. Studies comparing selective and non-selective β-adrenoceptor blockade (Deacon & Barnett, 1976; Newman, 1976; Lager *et al.*, 1979) have shown no significant difference between the depth of hypoglycaemia produced on either drug. Our finding of a lower nadir on atenolol does not agree with this, but the result only just achieved statistical significance ($P = 0.05$). In the absence of further evidence no real conclusion can be drawn.

We have demonstrated clearly that the cAMP response to hypoglycaemia is abolished by propranolol but unaffected by atenolol. This implies a β_2-adrenergic mechanism for the increase in cAMP. This is in agreement with the finding that the catecholamine sensitivity of adenyl cyclase in functional human liver shows an order of potency typical of a β_2-adrenoceptor (Pecker *et al.*, 1979). However, Strange & Mjos (1975) have shown that functional hepatectomy of rats abolished the rise in plasma cAMP occurring in response to glucagon administration but did not decrease the rise after isoprenaline. Thus the source of the plasma cAMP cannot be assumed to be hepatic. Although it has been shown in man that the liver will release cAMP in response to glucagon (Liljenquist *et al.*, 1974) further studies are necessary on its response to adrenergic stimulation.

Endogenous opiates may affect glucose homeostasis at both central and peripheral sites. β-endorphin has been shown to stimulate the central sympathetic outflow in rats resulting in a very marked rise in circulating adrenaline concentrations and a smaller rise in both noradrenaline and dopamine (Van Loon *et al.*, 1981). At the level of the pancreas itself specific β-endorphin immunofluorescence has been localised within human pancreatic islets (Bruni *et al.*, 1979), and β-endorphin has been shown to stimulate insulin and glucagon secretion in man (Reid & Yen, 1981). It was therefore of great interest to see whether opiate receptor blockade in man would affect glucose counter-regulation after hypoglycaemia. Naloxone did not have any significant effect on either the cardiovascular or hormonal responses to hypoglycaemia. In this study naloxone was given in the large dose of 10 mg over 130 min. This dose would be expected to have a powerful effect in blocking opioid receptors although the effect would be particularly pronounced against μ rather than δ receptors. Thus it appears that endogenous opioids are not powerful modulators of either catecholamine secretion or glucose homeostasis, although subtle effects may have been missed.

In conclusion we have shown that the rise in plasma cAMP occurring after hypoglycaemia is produced by

β^2-adrenergic receptor stimulation, although blockade of this rise by propranolol did not have a pronounced effect on glucose counter-regulation. Endogenous opiate receptor blockade with naloxone has no major effect on glucose homeostasis after hypoglycaemia.

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