THE EFFECT OF ATROPINE ON INSULIN RELEASE CAUSED BY INTRAVENOUS GLUCOSE IN THE RHESUS MONKEY

By

P. M. Daniel and J. R. Henderson

ABSTRACT

Glucose was given as a rapid intravenous injection to fasted anaesthetized rhesus monkeys, and the concentrations of blood glucose and plasma insulin were measured. Glucose caused a biphasic insulin response. The first phase (which was short) occurred 1 min after the injection; the second was longer, and occurred 17 to 30 min after the injection. Following the control glucose injection, each animal was given atropine, and the insulin response to an identical glucose stimulus was measured. This produced a mean reduction of 45% in the area under the curve of insulin release. Both phases of insulin release were inhibited by atropine. When glucose was given alone, its mean half-time of disappearance from the circulation ($t_{1/2}$) was 25.4 ($\pm$ 1.9 SEM) min. After atropine the mean $t_{1/2}$ was 36.6 ($\pm$ 3.1 SEM) min, a significant difference. It is suggested that the delayed rate of disappearance of glucose from the circulation is the result of inhibition of insulin release by atropine.

The islets of Langerhans have both adrenergic and cholinergic innervation (Esterhuizen et al. 1968) and this innervation is, in most species more extensive than that of the exocrine part of the pancreas. Evidence for the functional
activity of a cholinergic system in the living animal is provided by various experiments. Stimulation of the distal cut end of the sub-diaphragmatic vagus produces an increase in the concentration of circulating immuno-reactive insulin in the dog (Frohman et al. 1967; Kaneto et al. 1967) and in the baboon (Daniel & Henderson 1967); furthermore, in the isolated islet preparation, insulin release has been demonstrated in response to carbamyl choline, an effect which can be blocked with atropine (Malaisse et al. 1967). Thus the insulin releasing mechanism seems to have a cholinergic component.

The object of the experiments described here is to see whether atropine modifies the insulin response to intravenous glucose in the rhesus monkey. In the experiments described in this paper, glucose was given intravenously to anaesthetized animals, and the plasma insulin and blood glucose concentrations measured: the animals were then given atropine, and the response to an identical glucose load was again measured.

METHODS

Eleven rhesus monkeys (6 female and 5 male, weighing between 5.8 and 10.1 kg) were used in these experiments. The monkeys were fed on the mixed diet which is given routinely in these laboratories. They were fasted overnight, and anaesthetized on the morning of the experiment with phencyclidine hydrochloride (Sernylan®, Parke Davis) and a catheter tied into a femoral vein. Anaesthesia was maintained with pentobarbitone sodium (Martindale Samoore) given through the catheter, which was kept open throughout the experiment with a slow drip of 0.9% saline. Each animal was allowed to settle for half an hour after the catheter had been put in. Three resting samples of blood (each 1 ml) were then taken over 20 min, via a two way tap on the end of the catheter: 0.8 ml of each sample were put into tubes containing EDTA (for insulin assay) and 0.2 ml into tubes containing fluoride oxalate (for glucose estimation). As soon as the last resting sample was taken, glucose was given intravenously as a 25% solution at a dose of either 0.5 or 1.0 g/kg. This injection was given over 1 min.

Blood samples were taken at 1, 3, 5, 7, 10, 13, 16, 20, 25, 30, 40, 50, 60 and 90 min after the glucose injection. Atropine sulphate was then given intravenously. We found that the human dose of atropine (about 0.02 mg/kg) had no effect on heart rate or pupillary diameter of the monkeys, and that these effects could only be obtained by giving a dose of 0.5 mg/kg. Five min after its injection a resting blood sample was taken, and a glucose injection given exactly as in the first half of the experiment, followed by blood samples taken at the same intervals. Thirty ml of blood was thus taken in the course of each experiment.

Glucose concentrations were measured by an automated glucose oxidase technique and insulin by a double antibody immuno assay (Hales & Randle 1963). Human insulin was used as a standard; this is important, for the levels of insulin in these rhesus monkeys is consistently higher than the level that probably would have been found if rhesus insulin had been used as a standard, but rhesus insulin was not available.
Table 1.
Mean insulin concentration (µU/ml) following two different doses of glucose.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Resting level</th>
<th>Minutes after glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Large dose 1.0 g/kg</td>
<td>29.4</td>
<td>258</td>
</tr>
<tr>
<td>SEM (n = 4)</td>
<td>2.6</td>
<td>11.0</td>
</tr>
<tr>
<td>Small dose 0.5 g/kg</td>
<td>32</td>
<td>316</td>
</tr>
<tr>
<td>SEM (n = 5)</td>
<td>2.1</td>
<td>18.7</td>
</tr>
</tbody>
</table>

Significance of difference between means

\[ P<0.01 \quad 0.05 \quad 0.1 \quad 0.3 \quad 0.4 \quad 0.1 \quad 0.4 \quad 0.5 \quad 0.5 \quad 0.3 \quad 0.1 \quad 0.3 \quad 0.1 \]

Not significant
The response of plasma insulin to a single rapid iv injection of glucose. Arrow shows glucose injection. The top curve is the control (mean of 11 animals). The lower curve (mean of 9 animals) shows the response in the same animals after atropine. Each vertical line corresponds to twice se, and the difference between the two curves is significant to \(< 0.05\) for all pairs of samples except those at 5 min.

**RESULTS**

*The effect of glucose on insulin concentration*

The most striking feature of the response to a single intravenous glucose injection was the biphasic shape of the plasma insulin curve. The mean values of the insulin concentrations is shown in the upper curve of Fig. 1. The first
peak reaches its highest level of insulin 1 min after the end of the glucose injection; the level then falls to reach a nadir at 7 min, rises again, and the second (and major) phase of insulin release has a sustained peak at 17–30 min.

In 5 animals a low glucose dose (0.5 g/kg) was used, and in 6 a high (1.0 g/kg) dose. Both doses produced statistically indistinguishable insulin responses (see Table 1). It is assumed therefore that both stimuli are maximal, and the results from the 2 groups have been pooled in Fig. 1.

The effect of atropine on insulin concentration

Atropine had no effect on the resting levels of glucose or insulin in any animal, but it reduced the insulin released in response to glucose in every experiment. The effect of atropine on insulin release in all the animals is seen in Fig. 1, while Fig. 2 shows the result of a single experiment. The areas of all insulin curves were measured by counting the number of squares above the baseline between 0 and 90 min. The mean value for insulin areas produced in response to glucose alone (9 animals, 11 experiments) was 10 850 (± 1100 SEM) µU·ml⁻¹·min, and the response to glucose following atropine (9 animals, 11 experiments) was 3500 (± 1300 SEM) µU·ml⁻¹·min. The mean difference was 7350 (± 1200 SEM) µU·ml⁻¹·min, which is statistically significant (t = 4.7, P < 0.001).

Glucose injection produces a biphasic insulin response. The insulin concentration reached at the second peak has been plotted against that at the first peak in each experiment. Controls ○; atropine treated animals ●; r = 0.74; t = 4.7; P < 0.001.
The glucose and the rate of glucose absorption were also followed atropine, was 5900 (± 725) μU·ml⁻¹·min. This is a highly significant difference (P < 0.001) and represents a 45.6% reduction in the insulin area of the animals treated with atropine. This does not inevitably mean that atropine produced a 45.6% reduction in insulin release, although this seems the most likely explanation.

The effect of atropine on the two peaks

It can be seen from Fig. 2 that the insulin concentration at the first and second peaks was similar, irrespective of whether or not atropine had been given. In Fig. 3 the height of the second insulin peak has been plotted against the height of the first for each experiment and it can be seen that atropine reduces the height of both peaks equally.

The effect of two consecutive glucose injections without atropine

We have assumed that in a given experiment the second glucose stimulus, without atropine, would produce an insulin response equal to the first response. To test this assumption, 3 monkeys (2 of them were used on other occasions for the atropine experiments) were given two consecutive injections, but with no atropine preceding the second dose of glucose (2 of the animals were given 1.0 g/kg injections: one was given 0.5 g/kg). The insulin areas produced by the second stimulus showed changes of −14, −6 and +15% in the three experiments, which make it very unlikely that exhaustion of β cells played any part in the diminished insulin response seen after atropine.

Atropine and glucose concentrations

The rapid intravenous injection of glucose produced an abrupt peak in plasma glucose concentration followed by a rapid fall lasting 8–9 min. This we assume to be due to the mixing of glucose throughout the extracellular space, and also to the passage of glucose into cells. There follows a slower fall, beginning at 10–12 min after injection, when glucose had been fully mixed throughout the extracellular space, and any loss from the circulation must have been due to its passage into cells and its loss in urine. A typical experiment is shown in Fig. 4, which shows the two phases of fall on a semilogarithmic plot. The rate at which glucose passes into cells during the second phase is presumed to be dependent on insulin, so that if insulin release were inhibited by atropine, the rate of disappearance of glucose should be decreased by the drug following the second glucose injection. In other words, atropine should increase the glucose half-disappearance time (t½) following the second glucose injection. The glucose t½ was measured, for the period 12–60 min, after glucose alone and after atropine plus glucose. It may be seen from Table 2 that atropine produced a significant increase (45%) in the t½ of glucose, while simulta-
The rate of disappearance of glucose from the plasma in a single experiment. Control ●; after atropine ○. The slope of the line in the control corresponds to a disappearance time, $t_{1/2}$, of 23 min; that after atropine to a $t_{1/2}$ of 40 min. Glucose injected at arrow.

Fig. 5.
The relationship between the glucose disappearance time ($t_{1/2}$) and the area beneath the insulin curve between 0 and 90 min for each experiment. Control ○, atropine treated animals ●; $r = 0.54$; $t = 2.45$; $P < 0.0125$. 

742
Table 2.
The effects of atropine on glucose disappearance rates and on insulin area.
The numbers of experiments are in brackets.

<table>
<thead>
<tr>
<th></th>
<th>Control i.e. glucose alone</th>
<th>Atropine before glucose</th>
<th>Significance of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean insulin area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μU · ml⁻¹ · min) ± SEM</td>
<td>10 850 ± 1100 (11)</td>
<td>5900 ± 725 (9)</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Mean glucose t₁/₂ ± SEM (min)</td>
<td>25.4 ± 1.9 (11)</td>
<td>36.6 ± 3.1 (9)</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

neously producing a 45% reduction in insulin area. It is assumed that the reduction in insulin area (which represents reduced secretion) causes the increase in glucose t₁/₂, and there is in fact a significant negative correlation between insulin area and glucose t₁/₂ (Fig. 5). Glucose t₁/₂ values were not statistically different for the two doses of glucose given, and they have been pooled in Table 2. In the control animals (i.e. those monkeys given two glucose injections but no atropine) there was no significant difference between the t₁/₂ values measured after the first and second glucose injections.

**Discussion**

The biphasic insulin response seen in all the experiments described here is not a new phenomenon. Such a response was first described in glucose-induced insulin release from the isolated perfused rat pancreas (Grodsky et al. 1963, 1970; Pfeiffer et al. 1970) and from isolated islets of Langerhans (Cerasi & Luft 1972). As far as we are aware, a biphasic response has not previously been described in peripheral blood of the whole animal; however, few animals respond to intravenous glucose by secreting so large an amount of insulin as does the rhesus monkey (Wherry et al. 1966; Wilson & Martin 1970). Furthermore, in the experiments described here, the relatively large stimulus of a dose of glucose given over the short period of 1 min overcomes any tendency that a slower injection might have to blur the two phases of release; early, frequent sampling is essential if the two phases are to be revealed.

In man, when two consecutive doses of glucose (oral or intravenous) are given, the tolerance for the second dose increases, i.e. the rate of disappearance of glucose increases (Metz & Friedenberg 1970). The phenomenon has been called the Staub-Traugott effect; no obvious explanation for it exists, for the insulin release after the second injection of glucose is very similar to the first. However, the findings described in this paper show that in the
rhesus monkey the glucose tolerance decreased after the second glucose injection; the most reasonable explanation for this is that atropine inhibited insulin release, and that the diminished insulin release caused a decrease in glucose tolerance following the second glucose injection.

Some evidence pointing towards a cholinergic mechanism of insulin release has been cited already, and the subject has been well reviewed (Malaisse 1972). It is already known that atropine inhibits insulin release in experiments where bloodflow changes could play no part (i.e. the isolated islet preparation), so it would now seem reasonable to suppose that a significant proportion of the effect of atropine on the whole animal is the result of its direct effect on the islet β cell. But we have no knowledge of whether atropine might, for example, produce its effect by inhibiting vasodilator activity brought about by glucose, though this seems unlikely. Nor do we have any knowledge of whether atropine might exert its effect centrally as well as peripherally, for any cholinergic component of insulin release might involve a glucose receptor in the brain. But the results shown here (Figs. 4 and 5, Table 2) suggest that atropine inhibits the release of insulin into the blood, and that the diminished insulin release slows down the rate of disappearance of glucose from the circulation.

ACKNOWLEDGMENTS

We are grateful to the British Diabetic Association and the Research Funds of the Bethlem Royal and Maudsley Hospitals for grants which assisted this work.

REFERENCES


Received on May 6th, 1974.