vate the steroid-transforming enzymes. To correct for analytical losses, 0.025 μCi of [4-12,6,7-3H]androstenedione (specific radioactivity 84 Ci/mmol, Amersham International, Amersham, Bucks, U.K.) was added; the incubates were then extracted with ethyl acetate (2 × 2 ml). The pooled extracts were evaporated carefully to dryness under reduced pressure and the residue was dissolved in ethanol and purified by chromatography on Sephadex LH-20 (Kwan et al., 1988). Testosterone can be further reduced to isomer androstanoiols, i.e. 5α-androstane-3α, 17β- and 3β, 17β-diols. The formation of large yields of the odoriferous 16-androstenes, including 5α-androst-16-en-3β-ol, was also reported, and the possible significance of these has been discussed (Kwan et al., 1988; Kwan & Gower, 1988).

The effects of oxytocin on the formation of pregnenolone metabolites was variable, ranging from marked inhibition to pronounced stimulation. The formation of the following steroids was inhibited (percentage yields relative to those without oxytocin = 100%): 5α-androstane-3β,17β-diol (10); 4-androstenedione (10); 5α-androstane-3α,17β- plus 3β, 17β-diols (33); 5α-androst-16-en-3α- plus 3β-ols (25). Testosterone formation was only slightly reduced (80%) but, in contrast, that of 5α-androst-16-en-3-one was markedly stimulated (300).

The minor inhibitory effect of oxytocin on testosterone production is in keeping with infusion studies in man and macaque when plasma testosterone levels were not affected (Chiodera et al., 1984; Tan & Kwan, 1987). The results, however, conflict with those of Adashi & Hsueh (1981) and Kwan & Gower (1988) who found suppression of testosterone production by cultured rat testicular cells and microsomal fractions. It thus appears that there are important species differences to be considered with regard to the effects of oxytocin. Further, our present results suggest that oxytocin has quite profound inhibitory effects in the macaque testis on the 5-ene and 4-ene pathways for testosterone biosynthesis as shown by the marked inhibition of 5-androstene-3β, 17β-diol (90%) and 4-androstenedione (50%). It is conceivable that, since the former steroid is formed normally in exceptionally high yield from pregnenolone (Kwan et al., 1988), then there is still sufficient to maintain testosterone production in the presence of oxytocin, albeit at a slightly reduced level. Further metabolism of testosterone also seems to be inhibited, as evidenced by the diminished (33%) formation of isomer 5α-androstane-3β-diol. Such inhibition of the two pathways from pregnenolone and progesterone may result, however, in the alternative metabolic routes to 5,16-androstadien-3β-ol and 4,16-androstadien-3-one and thence to 5α-androst-16-en-3-one (pathways well-known in porcine testes, Gower, 1984). If so, this, together with the lowered metabolism to 5α-androst-16-en-3α- and 3β-ols, could explain the build-up of 5α-androst-16-en-3-one.

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Effects of α-adrenergic antagonists on insulin secretion from rat pancreatic islets

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Glucose-induced insulin secretion is inhibited by catecholamines, an effect which is mediated by activation of α2-adrenoceptors [1], and can be observed in vivo and in vitro [2]. In vivo, the inhibition of insulin secretion by catecholamines may be an important component of normal glucose homeostasis, but it may also contribute to the development of certain pathological states. For example, it has been suggested that certain types of non-insulin-dependent diabetes mellitus (NIDDM) could result from an increase in endogenous α2-adrenergic activity, contributing to defective insulin secretion [3]. Hence, α2-adrenergic antagonists are potentially useful adjuncts in the management of NIDDM. Indeed, DGS5128, a selective α2-adrenoceptor antagonist, is currently showing promise as a new oral hypoglycaemic agent [4]. Another α-adrenoceptor blocker, phentolamine, also increases glucose-induced insulin secretion in NIDDM [5]. However, it is unclear whether the ability of these agents to stimulate insulin secretion can be completely accounted for by their α2-adrenergic properties.

In the present study, we have investigated the effects of a range of α2-adrenergic antagonists on both basal and glucose-induced insulin secretion, to gain information on their possible mechanisms of action.

Islets of Langerhans were isolated from male Wistar rats by collagenase digestion [6]. Groups of three islets were incubated at 37°C in 500 μl of a bicarbonate-buffered medium, pH 7.4 [7], containing 4 mm-glucose, 1 mm-glutamine, 1 mm-serum albumin (1 mg/ml) and test reagents. After 60 min, samples were removed for measurement of insulin content by radioimmunoassay.

Abbreviation used: NIDDM, non-insulin-dependent diabetes mellitus.
Table 1. Effect of \( \alpha_2 \)-adrenergic antagonists on insulin secretion from isolated rat islets of Langerhans

Each value represents the mean rate of secretion ± S.E.M. Numbers of observations are shown in parentheses. *P < 0.025; **P < 0.005; ***P < 0.0005, all relative to 4 mM-glucose alone.

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>[Agonist] (M)</th>
<th>Incubation conditions...</th>
<th>4 mM-Glucose ± S.E.M.</th>
<th>20 mM-Glucose ± S.E.M.</th>
<th>20 mM-Glucose + noradrenaline (10(^{-5}) M) ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>—</td>
<td>47</td>
<td>0.46 ± 0.02 ± 0.07</td>
<td>2.03 ± 0.11*** (30)</td>
<td>0.57 ± 0.04 (30)</td>
</tr>
<tr>
<td>Phenolamine</td>
<td>10(^{-6})</td>
<td>11</td>
<td>0.41 ± 0.04 ± 0.07</td>
<td>2.16 ± 0.20 (11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10(^{-3})</td>
<td>12</td>
<td>0.61 ± 0.06* ± 0.10</td>
<td>2.31 ± 0.15 (10)</td>
<td></td>
</tr>
<tr>
<td>Benextramine</td>
<td>10(^{-6})</td>
<td>6</td>
<td>0.58 ± 0.10 ± 0.06*</td>
<td>2.38 ± 0.29 (12)</td>
<td>1.82 ± 0.15 (9)</td>
</tr>
<tr>
<td></td>
<td>10(^{-5})</td>
<td>12</td>
<td>0.61 ± 0.07* ± 0.10</td>
<td>2.38 ± 0.29 (12)</td>
<td>1.82 ± 0.15 (9)</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>10(^{-4})</td>
<td>22</td>
<td>0.63 ± 0.06* ± 0.10</td>
<td>2.38 ± 0.29 (12)</td>
<td>1.82 ± 0.15 (9)</td>
</tr>
<tr>
<td>Idazoxan</td>
<td>10(^{-4})</td>
<td>6</td>
<td>0.53 ± 0.04 ± 0.06*</td>
<td>2.38 ± 0.29 (12)</td>
<td>1.82 ± 0.15 (9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.56 ± 0.09 ± 0.10</td>
<td>2.38 ± 0.29 (12)</td>
<td>1.95 ± 0.05 (6)</td>
</tr>
</tbody>
</table>

All of the \( \alpha_2 \)-antagonists tested significantly relieved the inhibitory effect of noradrenaline (10\(^{-5}\)M) on glucose (20 mM)-induced insulin secretion (Table 1). When tested in the absence of noradrenaline, two of the agonists, yohimbine and idazoxan, did not stimulate insulin secretion, under either basal (4 mM-glucose) or stimulated (20 mM-glucose) conditions. In contrast, both phentolamine and benextramine significantly augmented the basal insulin secretion rate (see Table 1). This effect was dose dependent and required concentrations of each of the antagonists in the range 10\(^{-5}\) - 10\(^{-4}\) M. However, no stimulatory effect was seen when either of these antagonists was added to islets in the presence of a stimulating concentration of glucose (20 mM).

One possible explanation for the augmentation of basal insulin secretion by phentolamine and benextramine is that the islets contain endogenous noradrenaline which is released from nerve endings during the incubation period and inhibits insulin secretion by binding to \( \alpha_2 \)-receptors on the \( \beta \)-cells. Blockade of these receptors by the antagonists would then promote an apparent increase in secretion rate. This has been proposed as an explanation for the stimulatory effect of phentolamine on insulin secretion observed in normal mice in vivo [2]. However, this mechanism cannot explain the stimulatory responses observed in our study. If the increase in basal insulin secretion reflected antagonism of released noradrenaline then it would be expected that both yohimbine and idazoxan, which are highly selective \( \alpha_2 \)-antagonists, would also have promoted an increase in basal insulin secretion. This was not the case. Furthermore, phentolamine and benextramine should also have further enhanced the rate of insulin secretion when islets were incubated in the presence of 20 mM-glucose, since any inhibition by released catecholamines would be most evident under these conditions. No further enhancement of glucose-induced insulin secretion was observed.

On the basis of the present results it is not possible to provide a mechanistic explanation for the stimulatory effects of phentolamine and benextramine. We cannot even be certain that the response is directly related to interaction with the \( \alpha_2 \)-receptor, since it was only observed with antagonist concentrations at which the \( \alpha_2 \)-receptor site would be expected to be fully occupied. Further work will be required to resolve these issues.

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