The evaporation of sweat from the body surface is an important route of thermoregulatory heat loss in horses, particularly during exercise (Bonner, 1994; Carlson, 1994; Hodgson et al. 1994), and it was Langley and Bennett (1923) who first showed that intradermal injections of adrenaline elicited profuse but local sweating in these animals. It is now clear that this response is mediated by glandular \( \beta_2 \) adrenoceptors which allow adrenaline to evoke cyclic 3',5' monophosphate (cyclic AMP) formation, and that it is a rise in the circulating level of adrenaline that normally triggers the sweating response seen during exercise (Robertshaw and Taylor, 1969; Snow, 1977; Johnson and Creed, 1982; Bijman and Quinton, 1984). However, Langley and Bennett (1923) also noted that glands that had secreted in response to injected adrenaline failed to produce sweat when the animals were exercised later that day (see also Evans and Smith, 1956; Evans et al. 1957). The stimulated glands had thus entered a refractory state that lasted for several hours. Although it has since been shown that \( \beta_2 \) adrenoceptors lose their sensitivity to adrenaline during prolonged stimulation, this desensitisation usually reverses within 20–40 min of agonist removal (see e.g. Su et al. 1980; Levitzki, 1988; Hausdorff et al. 1990; Yu et al. 1993). There is, therefore, no obvious explanation for the persistent desensitisation seen in the equine sweat gland. The aim of the present study was to explore possible physiological factors responsible for this effect.

**Materials and methods**

**Cell culture**

Experiments were undertaken using a spontaneously transformed cell line derived from the secretory epithelium of the equine sweat gland (Wilson et al. 1993). Standard techniques were used to maintain these cells in William’s medium E supplemented with L-glutamine (1 mmol l\(^{-1}\)), penicillin (100 i.u. ml\(^{-1}\)), streptomycin (100 \( \mu \)g ml\(^{-1}\)), bovine insulin (5 \( \mu \)g ml\(^{-1}\)), epidermal growth factor (0.1 \( \mu \)g ml\(^{-1}\)), hydrocortisone (10 ng ml\(^{-1}\)), transferrin (5 \( \mu \)g ml\(^{-1}\)) and sodium selenite (5 ng ml\(^{-1}\)). For experiments, cells were removed from culture flasks using trypsin/EDTA, plated onto 24-well plates (approximately 5\( \times \)10\(^4\) cells well\(^{-1}\)) and allowed to grow into monolayers that essentially covered the base of each well (3–4 days).

**Measurement of cyclic AMP accumulation**

The culture medium was withdrawn from each well and the cells showed reduced responses to ATP and forskolin. Increased phosphodiesterase activity may thus contribute to the persistent desensitisation. Experiments using forskolin-preincubated (100 \( \mu \)mol l\(^{-1}\), 32 min) cells suggested that increased cytosolic cyclic AMP levels did not underlie the initial loss of sensitivity to adrenaline but that this second messenger may initiate the series of events leading to the generalised loss of sensitivity seen after 10 h.

Key words: sweat glands, cell culture, stimulus–secretion coupling, cyclic AMP, phosphodiesterases, IBMX, equine anhydrosis.
ethanesulphononic acid, 25, and d-glucose, 10 (pH adjusted to 7.4 with NaOH when bubbled with 5% CO₂/95% O₂). The cells were then incubated (10 min, 37°C) in this solution until experiments were initiated by adding drugs to the appropriate wells. After carefully measured time intervals, the salt solution was rapidly replaced by 0.5 ml of 0.1 mol l⁻¹ perchloric acid to arrest cellular metabolism and to extract cyclic AMP from the cells. The cyclic AMP content of each acid extract and the mass of protein in each well were then measured (Ko et al. 1994). All such measurements were made in duplicate or triplicate, and the results are expressed as pmoles of cyclic AMP per microgram of cellular protein (pmol µg⁻¹). Experimentally induced changes in cyclic AMP content were quantified by measuring the cyclic AMP content of stimulated cells and subtracting the basal cyclic AMP content, which was measured in unstimulated cells grown on the same plates. In some instances, the rate at which cyclic AMP had been exported from the cells was determined (pmol µg⁻¹ h⁻¹) by measuring the cyclic AMP content of the salt solution that had bathed the cells for 32 min. The effects of isobutylmethylxanthine (IBMX) were studied by dissolving this phosphodiesterase (PDE) inhibitor directly in the physiological salt solution. In all instances, the IBMX-treated cells were exposed to the substance for 10 min directly in the physiological salt solution. In all instances, the rate at which cyclic AMP had been exported from unstimulated (0.003±0.001 pmol g⁻¹) and in IBMX-treated cells. Forskolin-preincubated cells were studied by dissolving this phosphodiesterase (PDE) inhibitor directly in the physiological salt solution. In all instances, the IBMX-treated cells were exposed to the substance for 10 min before experiments were initiated by adding drugs to the appropriate wells.

Data analysis and experimental design

Preliminary experiments showed that there could be large variations in the magnitude of the responses seen in cells after different passage numbers, and so experiments were undertaken using strictly paired experimental designs in which control and experimental cells had been identically passaged and had been grown on the same plates under identical conditions. Data are presented as means ± standard errors, and values of N refer to the number of times a protocol was repeated using cells after a different number of passages. The significance of differences between mean values was evaluated using Student’s paired t-test. For some analyses, commercially available software (Grafit 3.0, Erithacus Software Ltd, Staines, UK) was used to fit sigmoid curves to experimental data sets using a least-squares regression procedure. Adrenaline-preincubated cells were exposed to the hormone (10 µmol l⁻¹) for 32 min at 37°C, washed with physiological saline and then incubated for carefully measured time intervals. Basal cyclic AMP levels and the increases in cellular cyclic AMP content evoked by adrenaline (10 µmol l⁻¹, 4 min), ATP (100 µmol l⁻¹, 16 min) or forskolin (100 µmol l⁻¹, 8 min) were then measured and compared with the responses evoked in control cells that had been grown on the same plates. To ensure that physically disturbing the cells did not affect their responsiveness, great care was taken to subject the control and experimental cells to the same pattern of washes/solution changes. The extent to which the preincubated cells had lost sensitivity to adrenaline (% desensitisation) was quantified using the expression:

\[
% \text{ desensitisation} = [1 - (E/C)] \times 100
\]

where \( E \) and \( C \) are the increases in cyclic AMP content evoked in the experimental and control cells, respectively. Initial experiments showed that cells did not tolerate prolonged incubation in physiological saline and so, in experiments involving recovery periods of longer than 1 h, monolayers were returned to complete culture medium once the desensitising stimulus was withdrawn.

To explore the effects of IBMX upon the extent to which desensitised cells recovered their sensitivity to adrenaline, adrenaline-preincubated cells were prepared as detailed above. These cells were then either allowed to recover under standard conditions or exposed to 5 mmol l⁻¹ IBMX for the final 10 min of the recovery period. Basal cyclic AMP levels and the response to adrenaline (10 µmol l⁻¹, 4 min incubation) were then measured for both groups of desensitised cells. As part of each experiment, adrenaline-evoked increases in cellular cyclic AMP content were also measured under control conditions and in IBMX-treated cells. Each experiment thus provided paired estimates of the desensitisation seen under control conditions and in IBMX-treated cells. Forskolin-preincubated cells were exposed to this substance (100 µmol l⁻¹) for 32 min.

Results

Cyclic AMP accumulation by control cells

Adrenaline consistently evoked cyclic AMP accumulation, and this response reached a clearly-defined peak after 4 min (Fig. 1). Once this peak was reached, however, cyclic AMP levels fell towards the basal value and, after 32 min, the response had decayed by 86.5±2.1% (Fig. 1). There was no significant difference between the rates at which cyclic AMP was exported from unstimulated (0.003±0.001 pmol µg⁻¹ h⁻¹) and adrenaline-stimulated (0.006±0.003 pmol µg⁻¹ h⁻¹) cells, and so it was clear that the falling phase of the response to

![Fig. 1. Time course showing the changes in cyclic AMP content that occur during exposure to 10 nmol l⁻¹ adrenaline. Values are means ± s.e.m., N=18; basal cyclic AMP content was 0.028±0.007 pmol µg⁻¹.](image-url)
Desensitisation in equine sweat gland cells

adrenaline was not due to an increased efflux of cyclic AMP. The magnitude of the response to adrenaline was concentration-dependent (Fig. 2, ●), and the concentration required to evoke a half-maximal response (EC50) was estimated to be 0.036±0.018 μmol l⁻¹. Although salbutamol, a selective β2 adrenoceptor agonist, also elicited cyclic AMP accumulation (EC50=0.164±0.036 μmol l⁻¹), the maximal response was only 33.9±1.5 % of that evoked by a maximally effective concentration (10 μmol l⁻¹) of adrenaline (Fig. 2A, ■). ICI-118,551 (Fig. 2B, ▲) and atenolol (Fig. 2B, ▼) caused concentration-dependent inhibition of the response to adrenaline, and the concentrations required to achieve half-maximal inhibition were estimated to be 1.2±0.2 nmol l⁻¹ for ICI-118,551 and 4.5±3 μmol l⁻¹ for atenolol. The cells also accumulated cyclic AMP when exposed to forskolin or ATP. The response to forskolin peaked after 8 min, but had decayed by only 15.1±10.4 % after 32 min. Even after 128 min, cellular cyclic AMP levels had only declined by 39±8.2 % (Fig. 3A). The response to ATP peaked after 16 min and had decayed by 5.4±12.0 % after 32 min and by 56.7±8.3 % after 128 min (Fig. 3B). These responses were thus more sustained than the response to adrenaline.

**Effects of IBMX**

Basal cyclic AMP content was unaffected by 1 mmol l⁻¹ IBMX (control 0.026±0.006 pmol μg⁻¹, N=4) but elevated (control 0.022±0.006 pmol μg⁻¹, IBMX 0.090±0.027 pmol μg⁻¹, P<0.05, N=4) by a higher concentration (5 mmol l⁻¹). Both concentrations potentiated the response to adrenaline by prolonging its rising phase, although this effect was more marked at the higher concentration (Table 1). However, once
forskolin-evoked (1 mmol l−1) measured the extent to which UTP (1 mmol l−1 uridine triphosphate (UTP), to inhibit adrenaline-evoked cyclic AMP accumulation (Wilson et al. 1996). To explore the possibility that this inhibitory pathway may be modified by adrenaline, we undertook experiments (N=4) in which we measured the extent to which UTP (1 mmol l−1) could inhibit forskolin-evoked (1 μmol l−1, 16 min) cyclic AMP accumulation in saline-preincubated and adrenaline-preincubated (10 min recovery) cells. In these experiments, the cyclic AMP content of unstimulated cells was 0.022±0.006 pmol μg−1 and this value was unaffected by preincubation in adrenaline (0.025±0.002 pmol μg−1). As anticipated (see Fig. 5A), 1 μmol l−1 forskolin evoked essentially identical responses in saline-preincubated (0.179±0.036 pmol μg−1) and adrenaline-preincubated (0.175±0.022 pmol μg−1) cells. Moreover, preincubation in adrenaline did not affect the inhibitory action of UTP (control 31.1±10 %, preincubated 34.0±8.0 %, N=5).

Table 1. Responses to 10 μmol l−1 adrenaline (4 min) were quantified in control cells and in cells that had been preincubated in 1 mmol l−1 or 5 mmol l−1 IBMX

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control (N=8)</th>
<th>1 mmol l−1 IBMX (N=4)</th>
<th>5 mmol l−1 IBMX (N=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.529±0.161</td>
<td>0.369±0.049</td>
<td>0.660±0.171</td>
</tr>
<tr>
<td>4</td>
<td>0.599±0.193</td>
<td>0.535±0.095</td>
<td>1.222±0.159*</td>
</tr>
<tr>
<td>8</td>
<td>0.512±0.110</td>
<td>0.582±0.103**</td>
<td>1.464±0.292*</td>
</tr>
<tr>
<td>16</td>
<td>0.249±0.111</td>
<td>0.470±0.060***</td>
<td>1.711±0.289**</td>
</tr>
<tr>
<td>32</td>
<td>0.070±0.045</td>
<td>0.158±0.029*</td>
<td>0.877±0.308*</td>
</tr>
</tbody>
</table>

Asterisks denote values that differed significantly from control (*P<0.05, **P<0.02, ***P<0.01).

Effects of preincubation in adrenaline

In initial experiments, cells were preincubated in 10 μmol l−1 adrenaline for 32 min and then allowed to recover for 2–42 min before basal cyclic AMP content and the response to a second exposure to 10 μmol l−1 adrenaline were measured. The data (N=5) suggested that preincubation in adrenaline evoked a fall in basal cyclic AMP content that occurred after 22 min (control 0.04±0.008 pmol μg−1, adrenaline-preincubated 0.026±0.005 pmol μg−1, P<0.05) and clearly showed that preincubation essentially abolished (desensitisation >95 %) the response to a subsequent application of adrenaline. Moreover, the cells did not recover their sensitivity to adrenaline within the time scale of this experiment. We therefore undertook further experiments in which the preincubated cells were allowed to recover for up to 56 h. This work did not confirm the fall in basal cyclic AMP levels described above, but verified the almost total loss of sensitivity to adrenaline. Moreover, these experiments showed that full recovery did occur (Fig. 4A) and that the half time for this process was 6.3±0.9 h (Fig. 4B). The properties of adrenaline-preincubated cells were further investigated in experiments in which adrenaline-preincubated cells were allowed to recover for 10 min or 10 h. As expected, the cells were essentially unresponsive to adrenaline after 10 min of recovery (Fig. 5A). However, these desensitised cells consistently showed enhanced responses to ATP, although the response to forskolin was normal (Fig. 5A). Earlier work established that cultured equine sweat gland cells express type P2U purine receptors, which allow certain nucleotides, including uridine triphosphate (UTP), to inhibit adrenaline-evoked cyclic AMP accumulation (Wilson et al. 1996). To explore the possibility that this inhibitory pathway may be modified by adrenaline, we undertook experiments (N=4) in which we measured the extent to which UTP (1 mmol l−1) could inhibit forskolin-evoked (1 μmol l−1, 16 min) cyclic AMP accumulation in saline-preincubated and adrenaline-preincubated (10 min recovery) cells. In these experiments, the cyclic AMP content of unstimulated cells was 0.022±0.006 pmol μg−1 and this value was unaffected by preincubation in adrenaline (0.025±0.002 pmol μg−1). As anticipated (see Fig. 5A), 1 μmol l−1 forskolin evoked essentially identical responses in saline-preincubated (0.179±0.036 pmol μg−1) and adrenaline-preincubated (0.175±0.022 pmol μg−1) cells. Moreover, preincubation in adrenaline did not affect the inhibitory action of UTP (control 31.1±10 %, preincubated 34.0±8.0 %, N=5).

Fig. 4. The increases in cellular cyclic AMP content evoked by 10 μmol l−1 adrenaline (4 min incubation) were quantified in cells that had been preincubated (32 min, 37 °C) in physiological saline containing 10 μmol l−1 adrenaline, washed with control saline and then allowed to recover, under control conditions, for up to 56 h. The time course of recovery is shown in A, where data for preincubated cells are plotted together with the results of directly analogous experiments undertaken using cells that had been grown on the same plates but preincubated in control saline. Values are means ± S.E.M. Pooled data from 11 such paired experiments are shown (●, adrenaline-preincubated cells; ■, control cells). The basal cyclic AMP content of the control cells was initially 0.014±0.003 pmol μg−1 and that of the adrenaline-preincubated cells was initially 0.016±0.002 pmol μg−1. These values did not change significantly throughout the experimental period. The effects of preincubation with adrenaline on the response to a subsequent adrenaline treatment are plotted against the recovery time in B.
Effects of IBMX upon adrenaline-evoked desensitisation

Adrenaline-preincubated cells were allowed to recover for 10 min before basal cyclic AMP levels and the response to a second, brief exposure (4 min) to 10 μmol l\(^{-1}\) adrenaline were quantified. Each such measurement was made both for cells that had been allowed to recover under control conditions and for cells that had been exposed to 5 mmol l\(^{-1}\) IBMX during the recovery period. The results of these experiments are presented in Table 2. The data confirm that IBMX raises basal cyclic AMP levels, both in saline-preincubated and in adrenaline-preincubated cells. Moreover, IBMX also potentiated the response to adrenaline in both groups of cells. However, the desensitisation seen in cells that had recovered under control conditions did not differ significantly from that seen in cells that had recovered in the presence of IBMX (Table 2). Table 2 also presents the results of experiments in which we explored

In each experiment, cells were preincubated (32 min, 37°C) in physiological saline or 10 μmol l\(^{-1}\) adrenaline and allowed to recover for 10 min or 10 h before the cyclic AMP content of unstimulated cells and the rise in cyclic AMP content evoked by a second 4 min incubation (37°C) in 10 μmol l\(^{-1}\) adrenaline were measured. These data are tabulated and were used to quantify the loss of sensitivity to adrenaline (% desensitisation).

For experiments involving 10 min of recovery, all measurements were made both for cells maintained under control conditions throughout the recovery period and for cells that had recovered in the presence of 5 mmol l\(^{-1}\) IBMX. In experiments involving 10 h of recovery, however, IBMX-treated cells were exposed to IBMX (5 mmol l\(^{-1}\)) for only the 10 min that immediately preceded the second exposure to adrenaline.

Experiments were undertaken using strictly paired protocols, and differences between mean values were evaluated using Student’s paired \(t\)-test. Asterisks denote significant effects of preincubation in adrenaline (* \(P<0.05\), ** \(P<0.01\), *** \(P<0.001\)) and after 10 h († \(P<0.05\), †† \(P<0.02\), ††† \(P<0.01\)).Basal cyclic AMP content was not affected by preincubation in adrenaline both after 10 min (control 0.028±0.005 pmol µg\(^{-1}\); pre-stimulated 0.021±0.008 pmol µg\(^{-1}\)) and after 10 h (control 0.030±0.009 pmol µg\(^{-1}\); pre-stimulated 0.035±0.016 pmol µg\(^{-1}\)) of recovery. Asterisks denote experiments in which the response of adrenaline-prestimulated cells differed significantly from that measured in control cells (* \(P<0.05\), ** \(P<0.02\), *** \(P<0.01\); Student’s paired \(t\)-test). Values are means ± s.e.m.
the effects of IBMX upon the desensitisation that persisted after 10 h. In these experiments, the recovering cells were maintained in culture medium until 10 min before the end of the recovery period, when they were transferred either to control saline or to saline containing 5 nmol l\(^{-1}\) IBMX. Basal cyclic AMP levels and the response to adrenaline were, once again, elevated by IBMX (Table 2), but analysis of the data from these carefully paired experiments showed that the 10 min incubation in IBMX significantly attenuated the desensitisation that persisted after 10 h (Table 2).

**Effects of preincubation in forskolin**

To explore the role of cyclic AMP in the desensitisation process, cells were preincubated (32 min, 37 °C) in 100 μmol l\(^{-1}\) forskolin and allowed to recover for 10 min or 10 h before basal cyclic AMP levels and the responses to adrenaline (10 μmol l\(^{-1}\), 4 min) and ATP (100 μmol l\(^{-1}\), 16 min) were measured. After 10 min (N=5), basal cyclic AMP levels were elevated (control 0.013±0.002 pmol μg\(^{-1}\); forskolin-stimulated 0.130±0.034 pmol μg\(^{-1}\), P<0.05) and the responses to adrenaline (control 0.397±0.069 pmol μg\(^{-1}\), preincubated 0.880±0.175 pmol μg\(^{-1}\), P<0.05) and ATP (control 0.235±0.051 pmol μg\(^{-1}\), preincubated 0.616±0.168 pmol μg\(^{-1}\), P<0.05) were potentiated. After 10 h (N=5), however, basal cyclic AMP levels were normal (control 0.011±0.003 pmol μg\(^{-1}\); forskolin-prestimulated 0.009±0.002 pmol μg\(^{-1}\)) and the responses to adrenaline (control 0.409±0.062 pmol μg\(^{-1}\); forskolin-stimulated 0.312±0.052 pmol μg\(^{-1}\), P<0.01) and ATP (control 0.242±0.049 pmol μg\(^{-1}\); forskolin-stimulated 0.085±0.029 pmol μg\(^{-1}\), P<0.001) were now reduced. Analysis of these data showed that preincubation in forskolin inhibited the response to ATP (67.8±6.3%) more effectively (P<0.001) than the response to adrenaline (24.4±4.3%).

**Discussion**

The magnitude of the response to adrenaline was concentration-dependent and the EC\(_{50}\) derived from the present data (approximately 35 nmol l\(^{-1}\)) was similar to the value obtained in an earlier study undertaken in the presence of a PDE inhibitor (approximately 45 nmol l\(^{-1}\); Wilson et al. 1993). The cells responded to the selective \(\beta_2\) adrenoceptor agonist salbutamol, although the maximal response was only approximately 35% of the maximal response evoked by adrenaline, suggesting that salbutamol acts as a partial agonist. The limited sensitivity to salbutamol, which has been reported in other cell types (O’Donnell, 1972; Grove et al. 1995), thus supports our earlier suggestion (Wilson et al. 1993) that these cells express \(\beta_2\) adrenoceptors. Further evidence for this came from the experiments using ICI-118,551 and atenolol. Both of these compounds could abolish the response to adrenaline, but ICI-118,551 was approximately 4000 times more potent. This compound preferentially blocks \(\beta_2\) receptors whereas atenolol is primarily a \(\beta_1\) antagonist, and so these data also indicate that the cells express \(\beta_2\) adrenoceptors. The present results, together with our earlier data (Wilson et al. 1993), therefore suggest that cultured equine sweat gland epithelial cells express the signal transduction pathway that allows adrenaline to control secretion *in vivo* (Robertshaw and Taylor, 1969; Snow, 1977; Johnson and Creed, 1982; Bijman and Quinton, 1984a,b).

The cells began to accumulate cyclic AMP as soon as they were exposed to adrenaline, but this initial rapid rise was followed by a decline that occurred despite the continued presence of agonist. The falling phase of this response was reduced, although not abolished, by the PDE-inhibitor IBMX. Studies of adipocytes (Pawson et al. 1974) provided an early indication that cyclic-AMP-mobilising agonists may also activate PDE, and it is now clear that the type 3 isoform of PDE (PDE-3) is activated by cyclic-AMP-dependent protein kinases (PKA). Increased PDE activity therefore accompanies a rise in cyclic AMP content in any cell type that expresses PDE-3 (Beavo, 1995; Erdogan and Houslay, 1997). However, the present experiments showed that the responses to forskolin and ATP were far more sustained than the response to adrenaline. It is, therefore, perfectly clear that the cells are able to maintain elevated levels of cyclic AMP for long periods. Moreover, IBMX did not altogether abolish the falling phase of the response to adrenaline, indicating that cyclic-AMP-evoked activation of PDE cannot be the only mechanism that underlies the fall in cyclic AMP levels seen in adenalinestimulated cells.

**Properties of adrenaline-preincubated cells**

Cells that had been preincubated in adrenaline became essentially insensitive to this hormone, and experiments in which adrenaline-preincubated cells were allowed to recover for 10 min showed that, initially, this desensitisation was not accompanied by reduced sensitivity to other cyclic-AMP-mobilising agonists. Indeed, the cells became more sensitive to ATP. It is interesting, in this context, that the equine epithelial cells also express P\(_{2}\Upsilon\) purine receptors (Ko et al. 1994) that allow nucleotides to inhibit cyclic AMP accumulation (Sipma et al. 1994; Schulze-Lohoff et al. 1995; Wilson et al. 1996). As these P\(_{2}\Upsilon\) receptors are co-expressed with the receptors that allow ATP to increase cellular cyclic AMP content (Ko et al. 1994), the magnitude of the ATP-evoked rise in cyclic AMP content will reflect the balance between the stimulatory and inhibitory pathways. The augmented responses seen in the desensitised cells raised the possibility that the inhibitory pathway may become downregulated in the adrenaline-preincubated cells, and this possibility was explored by measuring the extent to which UTP could inhibit the response to forskolin. UTP was used in these experiments as this nucleotide selectively activates the inhibitory pathway (Ko et al. 1994; Wilson et al. 1996). It was clear, however, that preincubation in adrenaline did not reduce the inhibitory effect of UTP, suggesting that an unknown mechanism enhances the effects of ATP upon the adrenaline-desensitised cells. The physiological basis of this novel effect is worthy of further study.
**Effects of IBMX upon acutely desensitised cells**

Experiments using adrenaline-preincubated cells that had been allowed to recover for 10h showed that the persistent desensitisation was accompanied by reduced sensitivity to other cyclic-AMP-mobilising agonists. This suggested that the initial desensitisation and the persistent desensitisation had different physiological bases. Further evidence for this came from the experiments in which cells were preincubated in forskolin. This initially failed to reduce the responses to adrenaline and ATP; in fact, these responses were markedly potentiated. However, forskolin is a membrane-permeant agent that activates adenylate cyclase without activating cell surface receptors (Seamon and Daly, 1981), and so it may be difficult to wash the cells entirely free of this substance. The augmented responses could, therefore, reflect the continued presence of a small amount of forskolin, and the elevated basal cyclic AMP levels in forskolin-preincubated cells are consistent with this. The important point to emerge from this experiment was that forskolin failed to mimic the initial desensitisation seen in adrenaline-preincubated cells. After 10h of recovery, however, forskolin-preincubated cells showed reduced responses to ATP and adrenaline. Increased levels of cyclic AMP thus appear to initiate a series of events leading, after 10h, to reduced sensitivity to cyclic-AMP-mobilising agonists. Experiments using IBMX showed clearly that increased PDE activity did not contribute to the loss of sensitivity seen after 10 min of recovery, but suggested that these enzymes may contribute to the persistent desensitisation seen after 10h.

Recent studies of Jurkat cells (Erdogan and Houslay, 1997) showed that forskolin initially evoked a small and transient rise in PDE activity caused by PKA-mediated activation of PDE-3. However, this small response was succeeded by a much larger increase that developed slowly and was due to increased expression of genes encoding the PDE-3 and PDE-4 isoforms. This became apparent after 2–5 h and reached a plateau after approximately 10h (Erdogan and Houslay, 1997). Similar, although less well documented, effects have been reported for several other cell types (see Beavo, 1995; Giembycz, 1996), and these cyclic-AMP-dependent increases in PDE expression seem to be mediated by transcription factors that are activated by PKA (Beavo, 1995; Giembycz, 1996; Erdogan and Houslay, 1997). Moreover, agonist-evoked increases in PDE-4 expression can contribute to the desensitisation of the β2 adrenoceptors in the immune and pro-inflammatory cells of the lung (see Giembycz, 1996).

**Biological significance of the present findings**

The present experiments showed that adrenaline-preincubated equine sweat gland epithelial cells became essentially insensitive to this hormone. Such desensitisation has been observed in many cell types and its mechanism extensively studied. It is now clear that an essentially ubiquitous enzyme, β-adrenoceptor kinase (β-ARK), selectively phosphorylates β-adrenoceptors that are occupied by agonist. Although these modified receptors can still bind adrenaline, the resultant hormone/receptor complex causes minimal activation of adenylate cyclase and so this phosphorylation process causes a progressive decline in adenylate cyclase activity during continuous stimulation. However, these modified receptors can be withdrawn from the plasma membrane by endocytosis, dephosphorylated and recycled to the cell surface by exocytosis. This allows desensitised cells to regain sensitivity once the desensitising stimulus is withdrawn. This recovery is usually complete within 20–40 min, and this capacity for rapid resensitisation is an important feature of β-ARK-mediated desensitisation (Su et al. 1980; Levitzki, 1988; Hausdorff et al. 1990; Yu et al. 1993). In the present study, however, several hours were required before the desensitised equine cells responded normally to adrenaline. These cells thus displayed unusually persistent desensitisation which was in excellent accord with the early observation made in vivo (Langley and Bennett, 1923). Moreover, the persistent desensitisation was, at least in part, sensitive to IBMX, suggesting a role for PDE, and this raises the possibility that cyclic-AMP-evoked increases in PDE expression (see Beavo, 1995; Giembycz, 1996; Erdogan and Houslay, 1997) may contribute to the persistent desensitisation seen in the equine sweat gland epithelium (Langley and Bennett, 1923; Evans and Smith, 1956; Evans et al. 1957).

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