centrations (300 μM compared with 30 μM in control rats) (Fig. 1b). Inhibition of 3-OHMeAP was negligible at 300 μM. In the subject treated with PB and phenytoin the pattern of inhibition was not different.

The increase in 4-OHAP formation after PB treatment in the rat is in agreement with the results of Kahn et al. (1992). The ratio of 4-OHAP/3-OHMeAP formation was also considerably higher in the subject treated with PB and phenytoin when compared with six other human samples from subjects not on drug therapy. Ketoconazole showed some selectivity in its pattern of inhibition in the rat, inhibiting antipyrine 4-hydroxylation more in the PB-induced rat than in the control rat. It was not possible to observe any selective effects of ketoconazole inhibition in man.

4-Hydroxyantipyrine and 3-hydroxymethylantipyrine were generous gifts from Dr. D. J. Back, University of Liverpool.


Received 21 March 1986

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Short-term hormonal regulation of amino acid transport in isolated hepatocytes

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Glucagon stimulates the transport of alanine into isolated hepatocytes in a biphasic manner. The initial protein synthesis-independent phase of stimulation occurs over a period of 30 min and has been suggested to be due to an increase in cell membrane potential (Edmondson & Lumeng, 1980). Glucagon is known to hyperpolarize the cell plasma membrane, as measured using microelectrodes in perfused liver (Friedmann & Dambach, 1973). This hormone also stimulates Na+/K+-ATPase activity in isolated hepatocytes (Hillenfeldt, 1981). This communication further investigates inter-relationships between these events.

Abbreviations used: SITS, 4-acetamido-4'-isothiocyanate-stilbene-2,2'-disulphonic acid; DIDS, 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid.

Isolated hepatocytes from starved rats were suspended in Krebs-Hensleit bicarbonate-buffered medium. The initial rate of alanine transport was measured over a 2 min time period, as described previously (McGivan et al., 1981). Chloride distribution was measured using 36Cl-, together with [3H]inulin, as a marker of the extracellular space. Na+/K+-ATPase activity was assayed as ouabain-sensitive uptake of 86Rb+.

The cell membrane potential in isolated hepatocytes has not so far been successfully measured by electrophysiological techniques or by the use of cationic dyes. The value for this membrane potential calculated from chloride distribution in isolated hepatocytes has been shown to be equivalent to the potential measured directly by microelectrodes in perfused liver under a variety of conditions (Bradford et al., 1985). Chloride appears to distribute passively across the cell membrane; inhibitors of chloride-transporting systems such as SITS DIDS and furosemide were found to be without effect on chloride distribution. It has also been shown that the (Na+/K+ + 2Cl-) co-transport system is absent in hepatocytes (Bakker-Grunwald, 1983). In the present study, cell membrane potential was therefore calculated from chloride distribution using the Nerst equation.

Table 1. Effects of various hormones on alanine transport, 36Cl- distribution and 86Rb+ uptake in isolated hepatocytes

<table>
<thead>
<tr>
<th>Additions</th>
<th>Initial rate of alanine transport (nmol/min per mg)</th>
<th>%Cl- distribution ratio</th>
<th>Membrane potential calculated from 36Cl- distribution (mV)</th>
<th>Rate of 86Rb+ uptake as a % of control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>1.90 ± 0.36 (4)</td>
<td>0.263 ± 0.011 (4)</td>
<td>−34.8 ± 1.08 (4)</td>
<td>100 (4)</td>
</tr>
<tr>
<td>Glucagon (300 nm)</td>
<td>2.40 ± 0.28 (4)*</td>
<td>0.211 ± 0.012 (4)*</td>
<td>−40.6 ± 1.50 (4)*</td>
<td>131.3 ± 9.7 (4)*</td>
</tr>
<tr>
<td>Cyclic AMP (0.1 ms)</td>
<td>2.47 ± 0.11 (4)*</td>
<td>0.123 ± 0.012 (3)*</td>
<td>−54.6 ± 2.47 (3)*</td>
<td>159.4 ± 22.9 (4)*</td>
</tr>
<tr>
<td>Vasopressin (25 μm)</td>
<td>1.82 ± 0.98 (3)</td>
<td>0.254 ± 0.008 (3)</td>
<td>−35.7 ± 0.75 (3)</td>
<td>111.6 ± 6.50 (3)</td>
</tr>
<tr>
<td>Phenylephrine (25 μm)</td>
<td>1.88 ± 0.40 (3)</td>
<td>0.260 ± 0.004 (3)</td>
<td>−35.1 ± 0.34 (3)</td>
<td>107.4 ± 1.54 (3)</td>
</tr>
</tbody>
</table>

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Table 1 shows that both glucagon and dibutyryl cyclic AMP stimulated the initial rate of alanine transport in hepatocytes. Both these effectors also induced a hyperpolarization of the cell membrane and an increase in Na⁺ / K⁺ -ATPase activity. The Ca²⁺ -dependent hormones, vaso-pressin and phenylephrine, at concentrations that influence metabolism in hepatocytes did not stimulate alanine transport and failed to affect the membrane potential or ATPase activity under the conditions used. The time courses of the stimulation of transport, membrane hyperpolarization and increase in Na⁺ / K⁺ -ATPase activity caused by cyclic AMP were similar. In each case a half-maximum effect was obtained after approx. 10 min and the effects were maximal 30–40 min after adding the effector.

The stimulation of alanine transport caused by cyclic AMP involved an increase in the Vₜₐ₉, of transport with no effect on the Kₘ for alanine. Alanine transport via both Na⁺ -dependent transport systems A and ASC was increased. Cyclic AMP also stimulated the Na⁺ -dependent transport of glutamine via System N. When the cell membrane potential was manipulated by titration with cyclic AMP or by the addition of high-K⁺ -containing medium, a good correlation between the rate of transport of alanine and the magnitude of the cell membrane potential was observed. Ouabain, which blocks Na⁺ / K⁺ -ATPase activity, greatly reduced the change in membrane potential caused by cyclic AMP and blocked the cyclic AMP-dependent simulation of transport.

These results are consistent with a mechanism in which glucagon, acting via cyclic AMP, causes a primary direct, or indirect, stimulation of the electrogenic Na⁺ / K⁺ -ATPase. The consequent hyperpolarization of the cell membrane increases the Vₜₐ₉ of the electrogenic Na⁺ -linked amino acid transport systems and thus stimulates amino acid transport. The exact nature of the mechanism by which glucagon and cyclic AMP increase the activity of the Na⁺ / K⁺ -ATPase is as yet unclear.


Received 21 March 1986

Interaction of atracurium with verapamil at the rat neuromuscular junction

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Atracurium besylate (Stenlake, 1979) is a new competitive, non-depolarizing, neuromuscular blocking agent. It has an advantage over the existing muscle relaxants in that it undergoes spontaneous degradation at physiological pH and body temperature. Thus it is suitable even in patients of atracurium with a Ca²⁺ -channel blocker, verapamil, was & advantage over the existing muscle relaxants in that it

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The preparation, rat phrenic nerve–hemidiaphragm, was set up in an organ bath containing 80 ml of Krebs–Henseleit solution maintained at 32 ± 2°C and bubbled with 5% CO₂ in O₂. The pH of the solution was kept at around 7.4. The motor nerve was repetitively stimulated at 0.2 Hz with a maximal voltage and 0.2 ms square pulse duration. Mechanical responses produced by electrical stimulation or by drug action were recorded isometrically, using a force transducer and a Washington pen recorder.

Atracurium (1-100 μg/ml) and verapamil (2-200 μg/ml) both reduced the amplitude of the twitch response in a dose-dependent manner. The mean IC₅₀ values (concentration to produce 50% maximum twitch inhibition) of atracurium- and verapamil-induced depression of twitch tension were: 10 ± 0.2 μg/ml and 40 ± 5 μg/ml respectively (n = 6, P < 0.001). Furthermore, verapamil (1-10 μg/ml) increased the neuromuscular blockade produced by atracurium (2-20 μg/ml). The mean IC₅₀ values of control tubocurarine-induced blockade were reduced by verapamil from 10 ± 0.2 μg/ml to 6.5 ± 0.5 μg/ml (mean ± S.E., n = 6, P < 0.05). Although the neuromuscular blockade produced by atracurium was easily reversed upon washing out the drug with Krebs solution, the combined blockade produced by atracurium and verapamil was difficult to reverse: it took 20–30 min. However, neostigmine (60 nM) and edrophonium (50 μM) both reversed the blockade produced by atracurium with or without verapamil. On the other hand, increasing the external Ca²⁺ concentration, from 2.5 mM to 5.0 mM, did not inhibit the depressant effect of verapamil, alone or in combination with atracurium. Since Ca²⁺ is needed for muscle contraction and neuromuscular transmission, it is possible that verapamil, by blocking the slow Ca²⁺ channels, at both pre- and post-junctional membranes, potentiates the neuromuscular blockade produced by atracurium. Thus the effects of atracurium and verapamil may be additive at both pre- and post-junctional membranes, albeit they are achieved via different mechanisms (Bikhazi et al., 1982; Wali, 1985a,b).


Received 22 January 1986