STEROIDOGENIC AND GLYCOLYTIC RESPONSES TO NUCLEOTIDES, NUCLEOSIDES AND STEROIDS IN RODENT ADRENAL GLANDS: OPPOSING, SPECIES-DEPENDENT EFFECTS OF CYCLIC GMP

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SUMMARY

The effects of nucleotides and nucleosides on steroidogenesis and aerobic lactic-acid production were examined in unsectioned mouse adrenal glands preincubated for 1 h and then incubated for 2 h in Krebs–Ringer bicarbonate and 0.01 m-glucose medium equilibrated with 95% O₂ : 5% CO₂. Of all the compounds tested, at a concentration of 10 mmol/l (cyclic AMP, cyclic GMP, AMP, ADP, ATP, GMP, IMP, adenosine, guanosine and inosine), only cyclic AMP was capable of stimulating steroidogenesis and induced a nine- to 12-fold increase in corticosterone production. Cyclic GMP inhibited corticosterone production by 40–55%. The nucleotides and nucleosides, except for ATP, all increased lactic-acid production. Cyclic AMP caused a three- to fivefold stimulation, cyclic GMP an increase of only 20–30%, and GMP, AMP and ADP increases of 80–100%. Cyclic GMP, protected from hydrolysis, may thus inhibit lactic-acid as well as steroid production in the mouse adrenal gland. By contrast, cyclic GMP was nearly as effective as cyclic AMP in stimulating glycolysis and steroidogenesis of rat adrenal glands. The proportion of corticosterone to 18-hydroxydeoxycorticosterone (18-OH-DOC) obtained with cyclic GMP was, however, always lower than that obtained with cyclic AMP. Cyclic AMP, as opposed to cyclic GMP, increased the formation of corticosterone and lactic acid in the presence of exogenous deoxycorticosterone (DOC) beyond that expected from an additive response. Lactic-acid production was inhibited by 18-OH-DOC, a major secretory product of the rat but not the mouse adrenal. This steroid, furthermore, greatly reduced the stimulation of glycolysis evoked by added DOC, 11β-hydroxyprogesterone and corticosterone, facts that could account for the greater glycolytic activity of mouse compared with rat adrenals. The yield of corticosterone in the presence of added 11β-hydroxyprogesterone, but not of DOC, was also reduced by 18-OH-DOC, denoting a selective inhibition of 21-hydroxylation. A structural analogue of 18-OH-DOC, 18,20-cyclo-20,21-dihydroxypregn-4-en-3-one, added by itself stimulated lactic-acid production. Added in combination with other steroids, it specifically counteracted the inhibitory effect of 18-OH-DOC, a steroid of potentially adverse biological properties. Our results are compatible with the concept that adrenal aerobic glycolysis is to a significant extent, but not exclusively, steroid-mediated. The glycolytically active but steroidogenically inert nucleotides and nucleosides offer examples of a dissociation between the two events.

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INTRODUCTION

That adrenocorticotrophin (ACTH) stimulates not only steroidogenesis, but also aerobic lactic-acid production, was first established with the use of intact mouse adrenal glands in vitro (Birmingham, Huberman & Riven, 1969). In contrast with the steroidogenic response, the glycolytic response of the mouse adrenal to ACTH requires the presence of exogenous glucose, and the increment of lactic-acid formation is stoichiometrically accounted for by the disappearance of glucose from the medium (Bartova & Birmingham, 1971). The production of lactic acid in this preparation is also stimulated in a dose-dependent manner by cyclic AMP and its dibutylr derivative, and by corticosterone, 11-deoxycorticosterone (DOC), 11β-hydroxyprogesterone and progesterone, provided the steroids are added in sufficiently high concentrations to induce a rise in the adrenal tissue content of corticosterone comparable to that evoked by ACTH (Bartova, Tibagong & Birmingham, 1971). The glycolytic effects of ACTH and cyclic AMP may therefore be to a significant extent steroid-mediated. In the present work, the response by mouse and rat adrenal glands to a variety of nucleotides, nucleosides and steroids has been examined in order to obtain more information on their role in controlling adrenal glycolysis and steroidogenesis, and the extent to which these two phenomena might be causally related.

MATERIALS AND METHODS

The experiments were performed on adrenal glands from 22-day-old Swiss albino mice (Charles River descendants) and from male Sprague–Dawley rats weighing 150 g, purchased from Canadian Breeders Ltd, La Prairie, Quebec, Canada. The animals were decapitated without anaesthesia, the adrenals were quickly removed, cleaned, left intact or quartered as specified, and samples of tissue weighing approximately 25 mg (20 intact mouse adrenals; two intact rat adrenals; or eight rat adrenal quarters) were preincubated for 1 h in 2 ml Krebs–Ringer bicarbonate and 0.01 M-glucose solution equilibrated with 95% O₂ : 5% CO₂ in a Dubnoff incubator. The tissue was transferred to 2 ml fresh medium and incubated for 2 h with the following additions where indicated: cyclic AMP, cyclic GMP, AMP, ADP, GMP, IMP, adenosine, guanosine and inosine (Sigma Chemical Company, St Louis, Missouri, U.S.A.) added in final concentrations of 2–10 mmol/1; steroids (Mann Research Laboratories, New York, New York, U.S.A.; CIBA–GEIGY, Basle, Switzerland) in final concentrations of 0.05–0.2 mmol/1. Dioxane, used to dissolve the steroids, was present in a final concentration of 0.45% in experimental and control media in the experiments involving exogenous steroids. At the end of the incubation, the steroids were extracted with dichloromethane from the medium and in some experiments from the glands homogenized in 0.9%(w/v) NaCl solution and dried. Corticosterone was measured by fluorometry (Zenker & Bernstein, 1958), 18-hydroxydeoxycorticosterone (18-OH-DOC) by the Porter–Silber reaction (Silber & Porter, 1954; Birmingham & Ward, 1961), and lactic acid in the medium by the method of Barker & Summers (1941) after deproteinization with barium hydroxide and zinc sulphate. The results were evaluated statistically using Student’s t-test and, to establish the significance of a deviation from an additive response, by analysis of variance.

RESULTS

Effects of nucleotides and nucleosides on corticosterone and lactic-acid production by mouse adrenal glands

Corticosteroid production

Of the ten compounds tested at a concentration of 10 mmol/l, only cyclic AMP was capable of stimulating corticosterone production in mouse adrenal glands (Fig. 1). It induced a nine- to 12-fold increase over the unstimulated output, comparable to the effect obtained with
1 µm-ACTH. By contrast, cyclic GMP caused a consistent and statistically highly significant inhibition of corticosterone production ranging from 40 to 55%. The non-cyclic nucleotides were ineffective or caused slight inhibition, the nucleosides were without effect. Corticosterone production in the presence of the combined addition of cyclic AMP and cyclic GMP did not differ significantly from an additive response.

**Lactic-acid production**

Statistically significant increases in lactic-acid production by mouse adrenal glands were obtained with all nucleotides and nucleosides tested, except ATP, but they were most pronounced with cyclic AMP, which evoked a three- to fivefold increase over the unstimulated output. Cyclic GMP increased lactic-acid production by only 20–30%, in contrast with the corresponding non-cyclic nucleotide GMP, a potential conversion product, which doubled glycolysis, as did AMP and ADP. An increase of 40% was evoked by IMP. Lactic-acid production in the presence of both cyclic AMP and cyclic GMP was significantly below that expected from an additive response.

**Effects of cyclic AMP and cyclic GMP on rat adrenal glands**

**Steroid production**

In striking contrast to the opposing responses to cyclic AMP and cyclic GMP noted with mouse adrenal glands, both cyclic nucleotides stimulated steroidogenesis in rat adrenal glands (Fig. 2). Cyclic GMP was in some experiments as effective as cyclic AMP in
stimulating the production of 18-OH-DOC, a major secretory product of the rat, but not of the mouse adrenal gland. However, in all experiments conducted on quartered or intact glands, the ratio of corticosterone to 18-OH-DOC obtained with cyclic AMP was higher than that obtained with cyclic GMP and a statistically significant difference between the production of the two steroids only occurred in the presence of cyclic AMP.

Lactic-acid production

Cyclic GMP caused a twofold and cyclic AMP a two- to threefold increase in the production of lactic acid by rat adrenal glands.

Effects of cyclic AMP and cyclic GMP on steroid production and glycolysis in the presence of exogenous DOC

Although the classical site of action of cyclic nucleotides in steroidogenesis is considered to be on the conversion of cholesterol to pregnenolone, the different proportions of corticosterone to 18-OH-DOC obtained with cyclic AMP and cyclic GMP suggested some additional differential effect on later stages of steroid biosynthesis such as enhancement of 11-hydroxylation by cyclic AMP or of 18-hydroxylation by cyclic GMP. The response to nucleotides and exogenous DOC at suboptimal concentrations, alone or in combination, was therefore examined (Table 1). That cyclic AMP was indeed capable of enhancing 11-hydroxylation was apparent from the statistically significant potentiating effect on corticosterone formation obtained with the combination of DOC plus cyclic AMP in both mouse and rat adrenal glands (expts 1 and 2). In contrast, only an additive increase in corticosterone formation occurred with the combination of DOC plus cyclic GMP (expt 3). The yield of 18-OH-DOC was below that expected from an additive response when either nucleotide was incubated with DOC and in combination with cyclic GMP this deficit was statistically highly significant.

The combined addition of DOC and cyclic AMP resulted in a significant potentiation of lactic-acid production by mouse adrenal glands (expt 1).
Table 1. Effects of cyclic nucleotides on the production of steroids and lactic acid in the presence of exogenous deoxycorticosterone (DOC) (values are means ± S.E.M.)

<table>
<thead>
<tr>
<th>Additions</th>
<th>Lactic acid (µg/100 mg per 2 h)</th>
<th>Corticosterone (µg/100 mg per 2 h)</th>
<th>18-OH-DOC (µg/100 mg per 2 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt 1 Mouse adrenals (n = 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a None</td>
<td>82 ± 5</td>
<td>2.5 ± 0.1</td>
<td>5 ± 0.2</td>
</tr>
<tr>
<td>b 2 mM-cyclic AMP</td>
<td>113 ± 4**</td>
<td>3.7 ± 0.2***</td>
<td></td>
</tr>
<tr>
<td>c 0.1 mM-DOC</td>
<td>148 ± 5***</td>
<td>71.6 ± 2.2***</td>
<td></td>
</tr>
<tr>
<td>d 2 mM-cyclic AMP + 0.1 mM-DOC</td>
<td>199 ± 3***</td>
<td>86.0 ± 2.0***</td>
<td></td>
</tr>
<tr>
<td>a + d – b – c</td>
<td>20 ± 9†</td>
<td>13.2 ± 2.9††</td>
<td></td>
</tr>
<tr>
<td>Expt 2 Rat adrenals (n = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a None</td>
<td>72 ± 14</td>
<td>5.1 ± 0.6</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td>b 2 mM-cyclic AMP</td>
<td>95 ± 9</td>
<td>24.1 ± 2.8**</td>
<td>19.1 ± 2.7**</td>
</tr>
<tr>
<td>c 0.1 mM-DOC</td>
<td>78 ± 5</td>
<td>38.2 ± 2.3***</td>
<td>28.0 ± 1.0***</td>
</tr>
<tr>
<td>d 2 mM-cyclic AMP + 0.1 mM-DOC</td>
<td>98 ± 4</td>
<td>68.7 ± 2.6***</td>
<td>34.1 ± 1.9</td>
</tr>
<tr>
<td>a + d – b – c</td>
<td>4 ± 17</td>
<td>11.5 ± 4.5†</td>
<td>−7.5 ± 3.5</td>
</tr>
<tr>
<td>Expt 3 Rat adrenals (n = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a None</td>
<td>101 ± 4</td>
<td>1.6 ± 0.2</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>b 8 mM-cyclic GMP</td>
<td>144 ± 11*</td>
<td>14.1 ± 1.0***</td>
<td>12.4 ± 1.6***</td>
</tr>
<tr>
<td>c 0.05 mM-DOC</td>
<td>106 ± 5</td>
<td>22.6 ± 0.8***</td>
<td>18.7 ± 2.8***</td>
</tr>
<tr>
<td>d 8 mM-cyclic GMP + 0.05 mM-DOC</td>
<td>152 ± 2***</td>
<td>33.1 ± 2.4***</td>
<td>18.6 ± 0.8***</td>
</tr>
<tr>
<td>a + d – b – c</td>
<td>13 ± 4</td>
<td>0.8 ± 2.7</td>
<td>−10.4 ± 3.1††</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01; ***P < 0.001: compared with value in row a. † Deviation from an additive response significant at P < 0.05; †† deviation from an additive response significant at P < 0.01. Degrees of freedom: expt 1, 1 and 12; expts 2 and 3, 1 and 8. The s.e. for the difference between the sums of two means is shown.

Effects of combinations of steroids on adrenal lactic-acid production

Natural steroids

The weaker glycolytic response of rat compared with mouse adrenals to cyclic AMP and, as noted in earlier work (Bartova & Birmingham, 1971), to ACTH suggested species-dependent differences in the steroid-mediated component of glycolysis, as rat but not mouse adrenals secrete 18-OH-DOC. Previous investigations had indicated that 18-OH-DOC does not stimulate glycolysis (Bartova et al. 1971). The possibility that it might inhibit the glycolysis evoked by corticosterone and precursors to corticosterone was tested in experiments summarized in Fig. 3b. Added by itself, 18-OH-DOC slightly reduced lactic-acid production in mouse adrenals, by 12–21 µg/100 mg. In combination with DOC, 11β-hydroxyprogesterone or corticosterone, the inhibitory effect of 18-OH-DOC was much more pronounced, averaging reductions of 52, 86 and 70 µg/100 mg respectively. The conversion of 11β-hydroxyprogesterone, but not of DOC, to corticosterone was greatly decreased by 18-OH-DOC, suggesting a specific inhibition of 21-hydroxylation (Fig. 3c). Since earlier experiments had indicated that the glycolytic response to steroids depended upon an increase in the corticosterone content of the tissue (Bartova & Birmingham, 1971), the corticosterone content in the glands was measured as well. The inhibitory effect of 18-OH-DOC on lactic-acid production was accompanied by a significant reduction of tissue corticosterone only in the glands exposed to the combination of 18-OH-DOC and 11β-hydroxyprogesterone.

18,20-Cyclo-20,21-dihydroxyprogren-4-en-3-one

A byproduct of the organic synthesis of 18-OH-DOC, 18,20-cyclo-20,21-dihydroxyprogren-
Fig. 3. Effects of steroid combinations on lactic-acid production (a and b) by intact mouse adrenal glands in vitro (means ± S.E.M.; n = 4). Additions: first column in group of four columns, none; second and third columns, steroids added by themselves (10 μmol/l); fourth column, steroids added in combination. 18-OH-DOC, 18-Hydroxydeoxycorticosterone; 11-OH-P, 11ß-hydroxyprogesterone; DOC, deoxycorticosterone; B, corticosterone; Cyclo., 18,20-cyclo-20,21-dihydroxypregn-4-en-3-one; Aldo., aldosterone. In the experiments shown in the lower part of the figure, corticosterone was measured as well (c), except in the medium of the glands incubated with added corticosterone. (——) Corticosterone in the medium; (———) corticosterone in the glands. *P < 0.05; **P < 0.01; ***P < 0.001: compared with values obtained in the absence of added steroids (Student's t-test). †P < 0.05; ‡P < 0.01; ‡‡P < 0.001: significant deviation from an additive response (analysis of variance).

4-en-3-one, has the same stereochemical configuration as 18-OH-DOC but the epoxy-linkage between carbons 18 and 20 is replaced by a carbon–carbon bond (Li, Birmingham & Chan, 1976). In contrast to 18-OH-DOC, the 18,20-cyclo-analogue stimulated lactic-acid production, and was thus an example of a glycolytically active steroid not convertible to corticosterone (Fig. 3a). The combination of the analogue with glycolytically inhibitory (18-OH-DOC, aldosterone) or stimulatory (DOC) steroids gave an additive yield only in the case of aldosterone: lactic-acid production in the presence of the analogue plus 18-OH-DOC significantly exceeded that expected from a summation of the stimulatory and inhibitory responses and equalled that obtained with the analogue alone. The yield in the presence of the combination of DOC with the analogue was significantly lower than that anticipated from a summation of responses and equalled that obtained with DOC alone.

DISCUSSION

Generic differences in nucleotide response

Our results denote generic differences in the adrenal metabolism of two members from the...
same subfamily of rodents, the murinae: (a) a divergent effect of cyclic GMP, inhibitory in the mouse and stimulatory in the rat; (b) a quantitative difference in the glycolytic response to cyclic AMP and, as noted previously, to ACTH (Bartova & Birmingham, 1971). Corticosterone production was reduced by half in mouse adrenals and stimulated tenfold in rat adrenals exposed to cyclic GMP. The Yin–Yang hypothesis of opposing functions for these nucleotides (Goldberg, 1975) may thus apply to the mouse but not the rat, its closest phylogenetic neighbour. Although lactic-acid production in mouse adrenals was increased slightly by cyclic GMP, the potential conversion product, GMP, was four times as active. Cyclic GMP greatly reduced the yield of lactic acid, but not of corticosterone, obtained in the presence of cyclic AMP suggesting interference with a component of glycolysis not mediated by steroids.

The finding that glycolysis in the rat adrenal is not as effectively stimulated by cyclic AMP and ACTH as is that in the mouse adrenal agrees with the concept that adrenal glycolysis is to a significant extent steroid-mediated since it may be accounted for by our observation that 18-OH-DOC, a major secretory product of the rat, but not the mouse, inhibits adrenal lactic-acid production and reduces the stimulation induced by the addition of corticosterone and potential precursors to corticosterone.

**Nucleotide effects on steroid metabolism**

The higher proportion of corticosterone to 18-OH-DOC obtained with cyclic AMP suggested differential effects of the cyclic nucleotides on late stages of steroid synthesis. Evidence for a differential effect on 11-hydroxylation was indeed obtained in experiments utilizing exogenous DOC which was converted more efficiently to corticosterone in the presence of cyclic AMP, but not of cyclic GMP. The yield of 18-OH-DOC from the combined addition of DOC with either cyclic GMP or cyclic AMP was less than additive. With DOC plus cyclic GMP the deficit was highly significant and corresponded exactly to the nucleotide-induced formation of 18-OH-DOC from endogenous sources. Exogenous DOC thus appeared to inhibit the conversion of endogenous substrate to 18-OH-DOC, but not to corticosterone. This is difficult to envisage if both end-products are derived from a common endogenous immediate precursor in the same cytological location. Our findings are compatible with a compartmentalization of precursor pools as suggested by Goddard, Vinson, Whitehouse & Sibley (1980) and Sibley, Whitehouse, Vinson & Goddard (1980), or they could indicate different metabolic pathways leading to 18-OH-DOC and corticosterone. Potential alternatives to DOC as precursor may be entertained for corticosterone as well as for 18-OH-DOC. In the case of corticosterone, the alternative 11β-hydroxyprogesterone is known to occur in adrenal tissue (Heap, Holzbauer & Newport, 1966; Traikov & Birmingham, 1966; Holzbauer & Newport, 1969), and to be a more efficient exogenous precursor to corticosterone than is DOC (Kraulis & Birmingham, 1964). The alternative precursor for 18-OH-DOC, 18-hydroxyprogesterone, is also a ready exogenous substrate (Ward & Birmingham, 1962), but its endogenous presence remains to be established.

**Steroid effects on aerobic glycolysis**

The marked glycolytic response evoked by corticosterone, DOC and 11β-hydroxyprogesterone confirms previous findings from this laboratory (Bartova et al. 1971). The 18-oxygenated compounds were either ineffective or inhibitory when added by themselves. The addition of 18-OH-DOc in combination with DOC, 11β-hydroxyprogesterone, or corticosterone resulted in a highly significant deficit from an additive response, suggesting the displacement by 18-OH-DOC of the active steroids from some glycolytically relevant receptor site. The early experiments from this laboratory (Bartova & Birmingham, 1971) indicated that the glycolytic effect of steroids is proportional to the amount of corticosterone.
accumulated in the tissue as is their inhibitory effect on protein synthesis (Clayman, Tsang & Johnstone, 1970). In the present experiments, the accumulation of corticosterone in the glands incubated with DOC or corticosterone was not affected by 18-OH-DOC which thus appeared to interfere with the glycolytic response to corticosterone.

The 18,20-cyclo-analogue of 18-OH-DOC proved to stimulate rather than inhibit glycolysis when added by itself and thus provided an example of glycolytic activity in a steroid not capable of conversion to corticosterone. That the analogue could not be transformed to corticosterone during the incubation by opening of the 18,20-bond was verified by chromatography of the steroid extracts. The combination of the 18,20-analogue with 18-OH-DOC, aldosterone or DOC yielded additive effects on lactic-acid production only in the case of aldosterone. In combination with 18-OH-DOC the same response occurred as with the cyclo-compound alone, and in combination with DOC the same response occurred as with DOC alone. The most plausible explanation for these results would seem to be that 18-OH-DOC could no longer exert any inhibiting action in the presence of its analogue, and that the analogue did not interfere with the action of DOC. This would occur if the analogue were capable of displacing 18-OH-DOC from some site to render it inactive, but could not displace DOC. Selective interference by the 18,20-cyclo-analogue with the action of 18-OH-DOC would be of interest in view of the potentially adverse effects of 18-OH-DOC (Birmingham, Oliver, Bartova, Frei & Levy, 1979; Beauwens, Crabbé & Birmingham, 1980).

Interrelations between nucleotide- and steroid-mediated glycolysis and possible sites of action

With the exception of ATP, the non-cyclic nucleotides tested caused a prominent stimulation of aerobic glycolysis but either they did not affect, or they inhibited steroidogenesis and therefore provided examples for the dissociation of the two events. A probable site of their action is at the level of phosphofructokinase, a rate-limiting glycolytic enzyme in various tissues including the adrenal (Bell, Brooker & Harding, 1970), for which AMP and ADP have been shown to be positive effectors (Passonneau & Lowry, 1962; Underwood & Newsholme, 1965; Weidemann, Hems & Krebs, 1969; Hickman & Weidemann, 1973). Since the nucleosides also caused a slight but significant stimulation of glycolysis, they might have contributed to the glycolytic effects of AMP and GMP to the extent to which these compounds are hydrolysed by the adrenal gland. At a concentration of 10 mmol/l, AMP was only half as effective as cyclic AMP. Cyclic AMP therefore exerted a specific glycolytic effect not attributable to its breakdown to AMP. This could be a direct one such as the activation of a rate-limiting glycolytic enzyme (Passonneau & Lowry, 1962) or an effect on glucose transport, since exogenous glucose is required for a maximal glycolytic response to cyclic AMP (Bartova & Birmingham, 1971), or it could be steroid-mediated.

The manner by which steroids affect aerobic glycolysis is open to debate. Nuclear interaction and enzyme induction are unlikely to account for the in-vitro effects because they occur without delay. Since the glycolytic response to steroids also requires the presence of exogenous glucose, enhancement of glucose transport as well as activation of glycolytic enzymes are possible modes of action. Interaction with an allosteric receptor system could be direct or indirect, such as through reduction of ATP/ADP:AMP ratios resulting from steroid metabolism requiring energy-linked NADPH synthesis (Cammer & Estabrook, 1967) or from an inhibition of the NADH oxidase system (Yielding & Tomkins, 1959).

Functional role of aerobic glycolysis

The functional role of aerobic lactic-acid production associated with steroidogenic glands and the stimulation of glycolysis in response to their trophic hormones remains to be established. Since in mouse (but not rat) adrenal glands the steroidogenic response to ACTH

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Rodent adrenal responses to cyclic nucleotides

is not impaired by the absence of glucose from the incubation medium even though lactic-acid production no longer occurs under such conditions (Bartova & Birmingham, 1971), glycolysis would not seem to be an obligatory mechanism for an additional energy supply required in steroidogenesis unless it is involved in the synthesis and storage of steroid precursors. It might function in providing additional blood supply to facilitate hormone release. Lactic acid enhances blood flow in various tissues and both blood flow and the content of lactic acid in adrenal vein blood are increased by ACTH infusion in the rat (Bartova, Tibagong, Holzbauer & Birmingham, 1973). It is conceivable that steroids and other agents capable of altering lactic-acid production affect the coordinated functioning of a tissue in view of the recent observation of Spray, Harris & Bennett (1981) that gap junctional conductance is a simple and sensitive function of intracellular pH. In excitable tissue, gap junctions mediate intercellular transmission of electrical signals and in inexcitable cells they allow intercellular spread of nutrients and metabolites and may transmit chemical messages.

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REFERENCES


