[19] also indicate independent effects of gonadal and adrenal steroids on hepatic oxidative metabolism. These observations indicate that the effects of testosterone to promote hepatic demethylase activity are not the result of direct and independent actions on the liver. Thus, the mechanism of action of testosterone to enhance hepatic oxidative metabolism differs from that of several synthetic steroids, including cyproterone acetate and pregnenolone-16α-carbonitrile [20, 21], whose effects are fully manifested in hypophysectomized animals.

It is presently not known whether the effects of testosterone are mediated completely by the pituitary gland or if testosterone interacts with some pituitary-dependent factor at the hepatic cell. The results do, however, suggest an important role for the pituitary in the regulation of hepatic mixed-function oxidases. Studies are now in progress to identify the pituitary factor(s) involved and determine the mechanism(s) of interaction with testosterone.

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*Departments of Physiology and Biophysics and Biochemistry, West Virginia University Medical Center, Morgantown, W. Va. 26506, U.S.A.*

ROBERT E. KRAMER
JOHN W. GREINER
WILLIAM J. CANADY
HOWARD D. COLBY

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# ATP-induced changes in microsomal $Mg^{2+}$ levels and relationship to muscle contraction in isolated guinea-pig ileum

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ATP has either an excitatory (contractile) or inhibitory (relaxing) effect on smooth muscle depending on the tissue [1]. Daniel and Irwin [2] have suggested that the contractile effect of ATP may be due its ability to complex $Mg^{2+}$ in the cell membrane, thereby favoring $Ca^{2+}$ entry and contraction. The present study was performed in an attempt to test this hypothesis and to determine effects of adenine nucleotides on the $Ca^{2+}$ and $Mg^{2+}$ binding activity of the membrane fraction isolated from guinea-pig ileum.

Guinea-pigs, weighing 350–500 g, were sacrificed by a blow on the head, and immediately the muscle layer of the ileum was dissected by the method of Ambache [3]. The microsomal fraction was prepared by the method of Schneider and Hogeboom [4]. The homogeneity of the subcellular fractions was checked by electron microscopy and the activities of the following marker enzymes were measured; NADH-cytochrome *c* reductase [5], glucose-6-phosphatase [6] and 5′-nucleotidase [7]. $Ca^{2+}$ and $Mg^{2+}$ binding activity of the fraction was measured according to the procedure described by Carvalho and Leo [8]. Microsomes (1 mg/3 ml of medium) were incubated in medium containing 150 mM KCl, 5 mM $MgCl_2$, 0·1 mM $CaCl_2$ and Tris–HCl (pH 7·4) with or without nucleotides at 25° for 10 min. The suspension was centrifuged at 0–5° for 30 min at 105,000 *g*. Bound $Ca^{2+}$ and $Mg^{2+}$ were determined by atomic absorption spectrophotometry. Protein concentration was determined by the method of Lowry *et al.* [9].

The activities of the marker enzymes (Table 1) indicate a satisfactory purity of the microsomal fraction.

As shown in Fig. 1, the $Mg^{2+}$ binding activity of the microsomal fraction was significantly decreased by ATP and ADP at concentrations greater than 1 mM, while the $Ca^{2+}$ binding activity was significantly increased only by ATP at concentrations of 0·1–6 mM. 5′-AMP, 3′-AMP and adenosine did not have any effect on the binding activities. However, the decreasing effect of ATP on the $Mg^{2+}$ binding activity was significantly potentiated by 5′-AMP, but not by 3′-AMP or adenosine (Fig. 1). Similarly the effect of ADP was potentiated by 5′-AMP (Fig. 1). However, the increasing effect of ATP on the $Ca^{2+}$ binding activity was not affected by 5′-AMP, 3′-AMP or adenosine.

Previous studies [10, 11] in this laboratory demonstrated that among the adenine nucleotides only ATP and ADP produce contraction in isolated guinea-pig ileum and their contractile effects are specifically potentiated by 5′-AMP. It was suggested that this phenomenon may provide a clue to elucidate the contractile mechanism of ATP. The present study indicates that these pharmacological effects

Table 1. Marker enzyme activities of the subcellular fractions isolated from the muscle layer of guinea-pig ileum

| Marker enzymes | Microsome | Mitochondria |
|---|---|---|
| NADH-cytochrome *c* reductase (nmoles cytochrome reduced/min/mg protein) | 100 ± 12 | 961 ± 51 |
| Glucose-6-phosphatase ($\mu$moles $P_i$ released/hr/mg protein) | 1·60 ± 0·20 | 0·17 ± 0·05 |
| 5′-Nucleotidase ($\mu$moles $P_i$ released/hr/mg protein) | 11·3 ± 1·1 | 1·5 ± 0·1 |

The results are expressed as mean ± S.E.of five experiments.

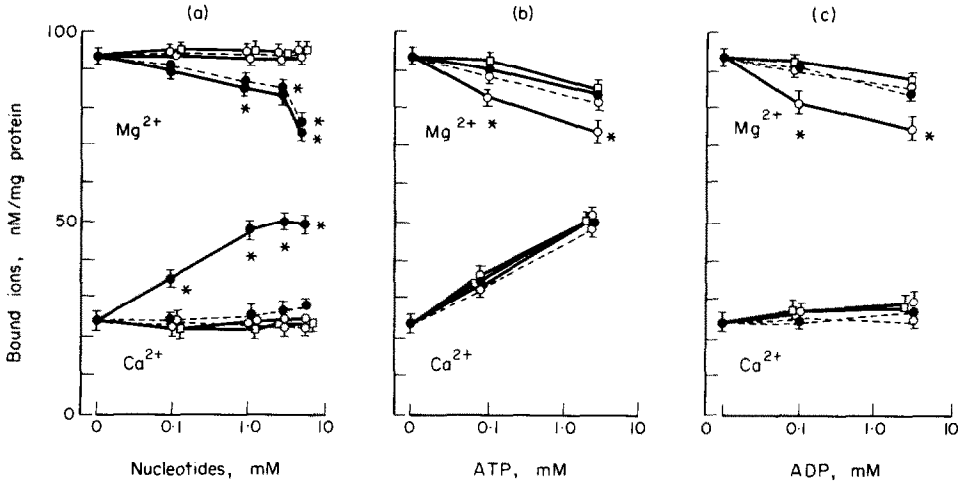


Fig. 1. $Ca^{2+}$ and $Mg^{2+}$ binding activity of the microsomal fraction isolated from guinea-pig ileum. (a) Effects of adenine nucleotides. ATP, ●——●; ADP, ●- - -●; 5′-AMP, ○——○; 3′-AMP, ○- - -○; adenosine, □——□. (b), (c) Effects of 5′-AMP, 3′-AMP and adenosine on the activity in the presence of ATP or ADP. ATP alone, ●——●; ADP alone, ●- - -●; with 6 mM 5′-AMP, ○——○; with 6 mM 3′-AMP, ○- - -○; with 6 mM adenosine, □- -□. Abscissa: log scale of millimolar concentration of the nucleotides added to the medium. The results are expressed as mean ± S.E. of six to ten experiments.
* $P < 0.05$ with respect to the control.

of the nucleotides are paralleled by a reduction in the $Mg^{2+}$ level, but not in the $Ca^{2+}$ level, in the microsomal fraction isolated from guinea-pig ileum; only ATP and ADP decrease the $Mg^{2+}$ binding activity of the fraction and their effects are potentiated by 5′-AMP. Since the nucleotides are known to form a complex with $Mg^{2+}$ rather than $Ca^{2+}$ [12, 13], the present findings support the theory of Daniel and Irwin [2] that the activity of ATP may be due to its ability to complex $Mg^{2+}$ present in the cell membrane, hence raising the $Ca^{2+}/Mg^{2+}$ ratio and favoring contraction. Moreover, it has been found that the bound $Ca^{2+}/Mg^{2+}$ ratio in the microsomal fraction from various tissues of guinea-pig is increased by incubation in medium containing ATP and the increment is more marked in excitatory tissues (ileum and vas deferens), which are contracted by ATP, than in inhibitory tissues (taenia coli and atria), which are relaxed [14]. The results suggest that changes in the microsomal $Mg^{2+}$ levels caused by ATP are involved in its pharmacological action on smooth muscle. The potentiation by 5′-AMP of the decreasing effect of ATP on the $Mg^{2+}$ binding activity is being studied.

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*Research Laboratory,* TADASHI ISO
*Santen Pharmaceutical Co., Ltd.,*
*Higashiyodogawa-ku, Osaka, Japan*

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