Effects of Mepacrine on the Oxidation of [1-14C]Oleate in Isolated Rat Hepatocytes

Masaru CHIHARA, Takahide NOMURA*, Masakatsu TACHIBANA, Hiroko NOMURA, Yukihiko NOMURA and Yasumichi HAGINO

Department of Pharmacology, Fujita-Gakuen Health University School of Medicine, Kutsukake-cho, Toyoake, Aichi 470-11, Japan

Accepted April 10, 1989

Abstract—In isolated rat hepatocytes, mepacrine stimulated the conversion of [1-14C]oleate into 14CO2 and depressed the formation of acid-soluble products from [1-14C]oleate. The action of mepacrine on [1-14C]oleate oxidation was not affected by exogenously applied phospholipase A2 (from Crotalus adamanteus venom) or arachidonic acid. It is suggested that mepacrine may exert its metabolic effects in isolated rat hepatocytes by a mechanism independent of phospholipase A2 inhibition.

Mepacrine exerts biological activities in platelets and isolated pancreatic islets, i.e., inhibition of platelet aggregation induced by thrombin (1) and suppression of glucose-induced insulin secretion (2). These mepacrine actions have been explained on the basis of the inhibition of cellular phospholipase A2 (3). Therefore, mepacrine appears to be a good probe for investigating the possible role of phospholipase A2 activation in hormone action. The action of vasopressin on isolated rat hepatocytes has been found to be associated with the release of arachidonic acid or its metabolites (4). We initially attempted to test whether mepacrine antagonizes vasopressin’s action on [1-14C]oleate oxidation in isolated rat hepatocytes, i.e., the stimulation of 14CO2 production from [1-14C]oleate (5–7) and the inhibition of the formation of acid-soluble products from [1-14C]oleate (6, 7). In the process of the experiments, however, we found that mepacrine itself produces marked effects on [1-14C]oleate oxidation in isolated rat hepatocytes.

Isolation and incubation of hepatocytes from fed rats, estimation of [1-14C]oleate oxidation and determination of acetoacetate and β-hydroxybutyrate were performed as described previously (6, 7). ATP was determined by the method of Lamprecht and Trautschold (8). The concentration of [1-14C]oleate (New England Nuclear, Boston, MA) in the incubation medium was 1 mM. Mepacrine dihydrochloride (Sigma Chemical Co., St. Louis, MO) and phospholipase A2 (from Crotalus adamanteus venom, Sigma) were dissolved in water before use. p-Bromophenacyl bromide (BPB, Katayama Chemical Industries Co., Osaka), indomethacin (Sigma), nordihydroguaiaretic acid (NDGA, Sigma), butylated hydroxytoluene (BHT, Aldrich Chemical Co., Milwaukee, WI), 1-phenyl-3-pyrazolidone (phenidone, Sigma) and arachidonic acid (Funakoshi Pharmaceutical Co., Tokyo) were dissolved in dimethyl sulfoxide. The results are expressed as means ± S.E.M. Statistical evaluation was made by Student’s t-test for paired data.

Mepacrine markedly stimulated [1-14C]oleate oxidation to 14CO2 and markedly depressed the formation of acid-soluble products (mainly ketone bodies) from [1-14C]oleate. The dose-response curve indicates that 0.5 mM mepacrine increased 14CO2 production to about 184% of the control (Fig. 1A) and depressed the formation of acid-soluble products to about 21% of the control (Fig. 1B). The effects of mepacrine (0.25 mM) on [1-14C]oleate oxidation were also

* To whom correspondence should be addressed.
produced in the absence of extracellular Ca\textsuperscript{++} (P<0.05) for \textsuperscript{14}CO\textsubscript{2} production: control, 0.85±0.03 μmol oleate utilized/30 min/g wet wt.; mepacrine, 1.28±0.03; for formation of acid-soluble products: control, 2.97±0.04; mepacrine, 1.24±0.07, N=4). Inhibition of ketogenesis from 1 mM oleate by mepacrine was confirmed by enzymatic determination of acetoacetate and β-hydroxybutyrate. Mepacrine (0.25 mM) reduced acetoacetate plus β-hydroxybutyrate by about 70% (P<0.05) (control, 20.6±1.2 μmol/30 min/g wet wt.; mepacrine, 6.1±1.0, N=4). Recently, Barritt et al. (9) reported that mepacrine, at a similar range of concentrations as in the present study, stimulated glycogen phosphorylase activity and calcium efflux in isolated rat hepatocytes.

Besides mepacrine, we also studied the effects of several other inhibitors of arachidonic acid metabolism. BPB, another inhibitor of phospholipase A\textsubscript{2} (1); indomethacin, a cyclooxygenase inhibitor (10); or an inhibitor of phospholipase A\textsubscript{2} (11); and two different lipoxygenase inhibitors, NDGA (12) and BHT (13), had no effect on \textsuperscript{1-14}C]oleate oxidation when tested at 0.25 mM concentration, respectively (Table 1). On the other hand, phenidone which inhibits both cyclooxygenase and lipoxygenase pathways (14) caused a dose-dependent stimulation of \textsuperscript{14}CO\textsubscript{2} production from \textsuperscript{1-14}C]oleate, although it failed to affect the formation of acid-soluble products from \textsuperscript{1-14}C]oleate (Table 1).

![Graph](image)

**Table 1.** Effects of BPB, phenidone, indomethacin, NDGA and BHT on \textsuperscript{1-14}C]oleate oxidation in isolated rat hepatocytes*  

<table>
<thead>
<tr>
<th>Additions</th>
<th>\textsuperscript{14}CO\textsubscript{2} production</th>
<th>Acid-soluble products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[\textsuperscript{1-14}C]oleate</td>
<td>% of control</td>
</tr>
<tr>
<td>Control</td>
<td>100 [1.01±0.03]</td>
<td>100 [3.90±0.26]</td>
</tr>
<tr>
<td>BPB 0.25 mM</td>
<td>104±6</td>
<td>89±2</td>
</tr>
<tr>
<td>Phenidone 0.1 mM</td>
<td>133±3*</td>
<td>103±2</td>
</tr>
<tr>
<td>Phenidone 0.25 mM</td>
<td>170±5*</td>
<td>105±3</td>
</tr>
<tr>
<td>Indomethacin 0.25 mM</td>
<td>107±1</td>
<td>97±1</td>
</tr>
<tr>
<td>NDGA 0.25 mM</td>
<td>108±8</td>
<td>95±5</td>
</tr>
<tr>
<td>BHT 0.25 mM</td>
<td>111±5</td>
<td>91±1</td>
</tr>
</tbody>
</table>

*Incubations were conducted for 30 min. Results are expressed as a percentage of the control value and are means±S.E.M. for three or four hepatocyte preparations. Absolute values are given in brackets for controls as means±S.E.M.; rates are expressed as μmol oleate utilized/30 min/g wet wt. Values that are significantly different from control incubations are indicated by *P<0.05.
BPB (0.25 mM), phenidone (0.25 mM), indomethacin (0.5 mM), NDGA (0.5 mM), BHT (0.5 mM) and mepacrine (0.25 mM) did not affect hepatocyte ATP content (control, 2.02±0.08 μmol/g wet wt.; BPB, 1.96±0.10; phenidone, 1.96±0.08; indomethacin, 1.97±0.07; NDGA, 1.95±0.10; BHT, 2.00±0.06; mepacrine, 2.12±0.13, N=4), suggesting that these agents exerted no toxic injury to hepatocytes.

To find out whether the actions of mepacrine and phenidone in isolated rat hepatocytes are associated with the inhibition of phospholipase A2 activity or that of arachidonic acid metabolism, the effects of exogenously applied phospholipase A2 and arachidonic acid were examined. Neither phospholipase A2 (1 U/ml) nor arachidonic acid (100 μM) antagonized mepacrine’s (0.25 mM) action on 14CO2 production (control, 1.16±0.03 μmol olate utilized/30 min/g wet wt.; phospholipase A2, 1.05±0.02; arachidonic acid, 1.16±0.04; mepacrine, 1.87±0.13; mepacrine plus phospholipase A2, 1.82±0.08; mepacrine plus arachidonic acid, 1.88±0.07, N=4) and the formation of acid-soluble products (control, 3.62±0.45 μmol olate utilized/30 min/g wet wt.; phospholipase A2, 3.79±0.34; arachidonic acid, 3.44±0.53; mepacrine, 1.45±0.13; mepacrine plus phospholipase A2, 1.51±0.11; mepacrine plus arachidonic acid, 1.46±0.31, N=4). The effect of phenidone on 14CO2 production was also not affected by these exogenously applied substances (results not shown). Our data suggest that mepacrine as well as phenidone may affect [1-14C]olate metabolism in the liver by mechanisms independent of their well-known effects on phospholipase A2 activity or arachidonic acid metabolism. Barritt et al. (9) documented that mepacrine stimulates inositol utilization in isolated rat hepatocytes. Furthermore, mepacrine has been shown to be a potent inhibitor of phosphatidic acid phosphatase (15) and acyl coenzyme A: lysocholcithin acyltransferase (16). Whether these actions of mepacrine are associated with the regulation of [1-14C]olate metabolism needs to be further investigated. Uncoupling of oxidative phosphorylation in the liver increases citric acid cycle activity (17), which prevents acetyl units generated by β-oxidation of olate from entering the ketogenic pathway and increases CO2 production. Mepacrine, however, is unlikely to act as an uncoupler in isolated rat hepatocytes since the agent did not affect whole-cell ATP content (described above).

References