Abnormalities of gallbladder muscle associated with acute inflammation in guinea pigs

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Xiao, Zuo-Liang, Qian Chen, Piero Biancani, and Jose Behar. Abnormalities of gallbladder muscle associated with acute inflammation in guinea pigs. Am J Physiol Gastrointest Liver Physiol 281: G490–G497, 2001.—Muscle strips from experimental acute cholecystitis (AC) exhibit a defective contraction. The mechanisms responsible for this impaired contraction are not known. The present studies investigated the nature of these abnormalities. AC was induced by ligating the common bile duct of guinea pigs for 3 days. Contraction was studied in enzymatic dissociated muscle cells. Cholecystokinin (CCK) and prostaglandin E2 (PGE2) receptor binding studies were performed by radioreceptor assay. The levels of lipid peroxidation, cholesterol, phospholipid, and H2O2 as well as the catalase and superoxide dismutase (SOD) activities were determined. PGE2 content was measured by radioimmunoassay. Muscle contraction induced by CCK, ACh, or KCl was significantly reduced in AC, but PGE2-induced contraction remained normal. GTPγS, diacylglycerol (DAG), and 1,4,5-trisphosphate (IP3), which bypass the plasma membrane, caused a normal contraction in AC. The number of functional receptors for CCK was significantly decreased, whereas those for PGE2 remained unchanged in AC. There was a reduction in the phospholipid content and increase in the level of lipid peroxidation as well as H2O2 content in the plasma membrane in AC. The PGE2 content and the activities of catalase and SOD were also elevated. These data suggest that AC cause damage to the constituents of the plasma membrane of muscle cells. The preservation of the PGE2 receptors may be the result of muscle cytoprotection.

Lipid peroxidation; free radical scavengers; cytoprotection

CALCULUS AND ACALCULUS acute cholecystitis (AC) is responsible for considerable morbidity and mortality (31). Acute acalculous cholecystitis is less common than acute calculous cholecystitis but has a rapid course and frequently results in complications and death. Between 6 and 17% of cases of AC occur in the absence of gallstones (45). The incidence as well as the surgical risk of this acute inflammatory condition increases with age, particularly in patients over 65, where the incidence is three times higher than in younger patients (10). The pathogenesis of human AC, however, has not been fully elucidated (17, 24). It has been suggested that the initial event is a transient obstruction of the cystic duct. The evidence supporting this hypothesis is circumstantial. Cystic duct ligation does not result in AC in animal models (45) unless the bile is supersaturated with cholesterol (38) or unless concentrated bile is introduced in the ligated gallbladder (44, 47). Furthermore, the contribution of the gallbladder mucosa and muscle in the pathogenesis of AC is not fully understood. Bile stasis frequently precedes the development of AC and could facilitate the diffusion of noxious agents (2). The mechanisms whereby these factors lead to AC are unclear.

The pathological changes seen in human AC with or without gallstones are similar (44, 45). These abnormalities can be reproduced in animals by ligating the common bile duct for 1 to 3 days (4, 31, 35). This model produces a picture of acute inflammation, which is pathologically indistinguishable from that seen clinically in acutely inflamed human gallbladders (31) (J. Behar, unpublished observations). Both specimens showed variable neutrophil and mononuclear infiltrates. Further, the guinea pig gallbladder muscle shares similar signal transduction pathways with human gallbladder (6, 7, 50–52). It is therefore a useful model for the study of the mechanisms of gallbladder muscle dysfunction induced by this acute inflammatory process.

The present studies therefore were aimed at determining the functional changes in gallbladder muscle cells induced by experimental acute inflammatory reaction in guinea pigs.

MATERIALS AND METHODS

AC model: common bile duct ligation. Ligation of the common bile duct for 3 days induced AC in guinea pigs (4, 35). The Animal Welfare Committee of Rhode Island Hospital approved their use. Adult male guinea pigs were purchased from Elm Hill Breeding Laboratory (Chelmsford, MA). They were housed in thermoregulated rooms with free access to food and water. After an overnight fast, guinea pigs were initially anesthetized with ketamine hydrochloride (30 mg/kg im) followed by an intraperitoneal injection of Nembutal (50 mg/kg). Additional Nembutal is given as needed to keep the animal deeply sedated. The animals were then placed on a warming pad of the surgical table. The entire procedure was

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performed under sterile conditions. The upper abdomen was shaved, scrubbed with betadine solution, and covered with sterile drapes. After a midline incision (2 cm), the gallbladder and pericholecystic region was carefully exposed. Then, the distal end of the common bile duct was identified and ligated at its junction with the duodenum using a 4–0 silk ligature (Biospec Products, Oklahoma, USA). The abdomen was closed in a two-layer closure and the surgical incision was cleaned and covered with sterile gauze and tape. Control animals were sham operated, which included all the surgical steps except for the common bile duct ligation. After the operative procedures, the animals were caged separately to recover from anesthesia and allowed to roam freely. They were then provided with food (laboratory chow) and water ad libitum for next 3 days. All guinea pigs received routinely analgesia with buprenorphine 0.05 mg/kg b.i.d. for the duration of the postoperative period. Three days later all animals were reanesthetized with ketamine followed by Nembutal (50 mg/kg ip) and killed for tissue harvest.

Isolation and permeabilization of muscle cells. Gallbladder muscle cells were obtained by a method that has been previously reported (52, 55–57). Briefly, after the gallbladder was removed, its mucosa was rinsed with ice-cold, oxygenated Kreb’s solution. The mucosa and serosa were carefully peeled off under a dissecting microscope. The muscle layer was cut into 2-mm wide strips and digested in HEPES buffer containing 0.5 mg/ml type I collagenase and 2 mg/ml papain (activity of ~13.9 U/mg protein) for 20 min at 35°C in a shaking water bath. The buffer was gently gassed with 100% O2 during digestion. At the end of the digestion, the tissue was filtered through a Nitex mesh 200 (Tetko, Elmsford, NY) and rinsed with 20 ml HEPES. The tissue remaining on the filter was collected and incubated in HEPES buffer at 35°C for 15 min to allow the free dispersion of cells, which were then ready for further use.

For the permeable preparation of muscle cells, the digested muscle tissue was rinsed with “cytotoxic buffer” [20 mM NaCl, 100 mM KCl, 25 mM NaHCO3, 0.96 mM Na2HPO4, 0.48 mM CaCl2, 5 mM MgSO4, 1 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid (EGTA), 2% bovine serum albumin (BSA)] (3, 52, 55–57). After the cells were dispersed, the cell suspension was briefly treated with saponin (75 μg/ml) by centrifugation at 200 g for 3 min. Cells were washed and resuspended in modified cytosolic buffer for further use.

Muscle cell contraction was induced in cell suspensions as described previously (3, 52, 55–57). They were allowed to react with the agonists for 30 s and then fixed by adding acrolein at a final concentration of 1%. Thirty consecutive intact cells were measured using a phase contrast microscope (Carl Zeiss, Oberkochen, Germany) and a television camera (Panasonic CCTV, model WV-CD51, Matsushita Communication, Osaka, Japan) connected to a Macintosh PowerPC computer. A computer software program (Image 1.33, NIH, Bethesda, MD) measured the cell length. Contraction was expressed as the mean of the percent shortening of 30 individual cells with respect to control (i.e., untreated) cells. Dose-response curves were constructed for cholecystokinin (CCK)-8, ACh, prostaglandin E2 (PGE2), KCl, GTPγS, diacylglycerol (DAG), and 1,4,5-trisphosphate (IP3) as agonists.

Preparation of enriched plasma membranes. Plasma membranes were prepared and purified by sucrose gradient centrifugation as described before (40, 51). Muscle cells were homogenized by using a tissue tearer (Biospec Products, Racine, WI) in 10 vol by weight of sucrose-HEPES buffer. It was further homogenized using a Dounce grinder (Whenton, Millville, NJ). The homogenates were centrifuged at 600 g for 5 min; the supernatant was collected in a clean centrifuge tube (Beckman Instruments, Fullerton, CA) and centrifuged at 150,000 g for 45 min to make a crude particulate pellet. The pellet was resuspended in sucrose-HEPES. This homogenate was then layered over a linear 9–60% sucrose gradient and centrifuged at 90,000 g for 3 h. The plasma membranes were collected at ~24% sucrose. They were then diluted and pelleted by centrifugation at 150,000 g for 30 min. The pellet of plasma membranes was stored at ~70°C.

Radioreceptor assay. Ligand binding experiments were performed in a final volume of 300 μl (39, 51). Membranes containing 50 μg of protein were incubated with 50 pM of [125I]-CCK-8 and unlabeled CCK (10–12 to 10–7 M) for 90 min or with 100 pM of [3H]PGE2 and unlabeled PGE2 (10–11 to 10–4 M) for 60 min at 25°C. Separation of bound from free radio-ligand was achieved by filtration utilizing a vacuum filtering manifold with receptor-binding filter mats (Millipore, Bedford, MA), and the filters were washed with ice-cold incubation medium without BSA. Radioactivity remaining on the filters was counted in a γ-scintillation counter for 125I or β-scintillation counter for 3H. The results are expressed as specific binding (%) achieved by subtracting nonspecific binding (assumed as the binding in the presence of 10–7 M unlabeled CCK or 10–4 M unlabeled PGE2) from total binding.

Analysis of data. Analysis of data was done using a computer analysis with a ligand fitting program (27) based on the radioreceptor assay data that analyzes the binding results to obtain the maximal specific binding capacity of CCK and PGE2 receptors.

Measurements of cholesterol and phospholipids. Lipids were extracted from purified plasma membranes with chloroform-methanol (54). After centrifugation at 2,000 g for 5 min, the lower organic phase was collected and equally dispersed to six glass test tubes for triplicate measurements of cholesterol and phospholipids. Samples were dried under a stream of nitrogen. The cholesterol content was measured with the cholesterol-oxidase method using a Sigma kit. One milliliter of cholesterol reagent was added to each dried sample and to 10-μl cholesterol standards (2 mg/ml), mixed, and incubated at 25°C for 10 min. They were measured at an optical density of 500 nm in a Beckman DU-7 spectrophotometer (Beckman Instruments, Palo Alto, CA). The cholesterol content was calculated according to the standards, calibrated by the protein content, and expressed as micromoles per milligram of protein.

Phospholipids were measured by previously reported methods (1, 54). Dried lipid extracts were digested with 0.5 ml of a 70% perchloric acid solution in a sand bath of 150°C until clear (~2 h). After the samples were cooled to room temperature, 4.6 ml of 0.22% ammonium molybdate and 0.2 ml of 16% Fiske-Subbarow reagent (Sigma, St. Louis, MO) were added to develop a color intensity proportional to the presence of inorganic phosphates. Samples were then boiled for 8 min and cooled for 20 min. They were detected at optical densities of 830 nm in a Beckman DU-7 spectrophotometer. The content of phospholipids was calculated according to the KH2PO4 primary standards and expressed as micromoles per milligrams of protein.

Assessment of lipid peroxidation. Purified plasma membranes were resuspended with 1.15% KCl (34) and mixed with 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid solution (pH 3.5), and 1.5 ml of 0.8% aqueous solution of TBA. This mixture was added to a volume of 4 ml with distilled water. The sample was heated at 95°C for 60 min. After cooling, 1 ml
of distilled water and 5 ml of the mixture of n-butanol and pyridine (15:1, vol/vol) were added and shaken vigorously. Spun at 2,000 g for 10 min, the organic layer was taken and its absorbency (red pigment) was measured at 532 nm. Tri-methoprim (TMP) was used as an external standard. The level of lipid peroxides was expressed as nanomoles of malonaldehyde (MDA, a secondary product of lipid peroxidation). Measurements were standardized to 100 mg of protein.

**Determination of H₂O₂ content.** It is based on the oxidation of ferrous ions to ferric ions by H₂O₂ (16). Under acidic conditions, ferric ions bind with the indicator dye xylene orange to form a stable colored complex that can be measured at 560 nm. H₂O₂ was measured in homogenized single gallbladder muscle cells from control animals and animals with AC. The production of H₂O₂ was determined by the H₂O₂-560 quantitative assay kit (Oxis International, Portland, OR). Briefly, H₂O₂ standard was prepared at a range of 0.1–100 μM. One volume of sample or standard was added to 10 vol of working reagent. This solution was mixed well and incubated at room temperature for 25–30 min, and the absorbency was measured at 560 nm. The content of H₂O₂ was calculated from the standard curve. The results were expressed as micromoles per milligrams of protein.

**Measurements of PGE₂ content.** The content of PGE₂ was measured by using a radioimmunoassay kit from NEN (NEN Life Science Products, Boston, MA; Ref. 13). Muscle cells were homogenized in HEPES buffer containing EDTA/indomethacin to inhibit the metabolism of arachidonic acid to prostaglandins. The suspension was centrifuged at 10,000 g for 15 min. PGE₂ was extracted from the supernatant by the methods of Kelly et al. (19). Extracted PGE₂ was measured by converting this prostaglandin into its methyl oximate derivative using the methyl oximation reagent. The determination of PGE₂ content was achieved by following the kit’s protocol. The content of PGE₂ was expressed as picomoles per milligrams of protein.

**Measurement of catalase activity.** The catalase activity was determined by using the method reported by others (8, 33). Muscle cells were homogenized in HEPES buffer. The suspension was centrifuged at 10,000 g for 15 min. Duplicate 25-μl aliquots of each sample of the supernatant and 75 μl of 10 mM phosphate buffer, pH 7.4, were placed into 3-ml cuvettes. Duplicate blanks (B) of solutions containing 90 μl of 0.01 N KMnO₄, 1 ml of 6 mM H₂O₂, and 1 ml of 6 mM H₂SO₄ were also prepared. The enzymatic reactions were initiated sequentially at fixed intervals by adding 1 ml of 6 mM H₂O₂ to the samples. After 3 min, the reactions were stopped sequentially at the same fixed intervals by rapidly adding and mixing 200 μl of 6 N H₂SO₄. Finally, 1.4 ml of 0.01 N KMnO₄ was added to each cuvette and mixed thoroughly. A spectrophotometric standard (S₀) was prepared by adding 7.0 ml of 0.01 N KMnO₄ to a mixture of 5.5 ml phosphate buffer and 1.0 ml of 6 N H₂SO₄. The absorbency of the solution was read at 480 nm within 30–60 s. One unit of catalase activity (k) was defined as the amount of enzyme that consumed 1 μmol H₂O₂·mg protein⁻¹·min⁻¹. Calculation of the catalase activity was achieved according to the equation: k = log (S₀/S₁) × 2.3/t. (S₀ was obtained by subtracting the absorbency of B from the S₀; S₁ was obtained by subtracting the absorbency of the samples from S₀; t = 3 min in this experiment). Data were expressed as units per milligrams of protein.

**Measurement of SOD activity.** The total SOD activity was measured by using a spectrophotometric assay kit (R&D systems, Minneapolis, MN; Ref. 32). Muscle cells were homogenized in HEPES buffer. The supernatant was obtained after the suspension was centrifuged at 8,500 g for 10 min at 4°C; 400 μl of ice-cold extraction reagent [ethanol/chloroform, 62.5:37.5 (vol/vol)] was added to 250 μl of the supernatant, vortexed for at least 30 s, and centrifuged at 3,000 g for 10 min at 4°C. Then, the aqueous upper layer was collected for assay of the SOD activity. The determination of SOD activity was achieved by following the kit’s protocol. Data were expressed as units per milligrams of protein.

**Protein determination.** The protein content of the muscle membranes was measured using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Melville, NY). Values for each sample were means of triplicate measurements.

**Drugs and chemicals.** ¹²⁵I-CCK-8, ³H-PGE₂, and PGE₂ radioimmunoassay kits were obtained from NEN Life Science Products. H₂O₂ quantitative assay kit was from Oxis International (Portland, OR). Type F collagenase, papain, cholesterol, TMP, thiobarbituric acid, pyridine, IP₃, GTPγS, DAG, and other reagents were purchased from Sigma.

**Data analysis.** One and two factorial repeated analysis of variance (ANOVA) and student’s t-test were used for statistical analysis. P < 0.05 was considered to be statistically significant.

**RESULTS**

The average resting lengths of intact and permeabilized smooth muscle cells isolated from sham-operated guinea pig gallbladders were 59.2 ± 1.1 and 58.7 ± 0.9 μm (n = 3), respectively. In animals with AC, it was 55.8 ± 1.2 and 57.4 ± 1.9 μm (n = 3), respectively. No significant differences in resting cell length were observed between these two groups (one-factor ANOVA).

Membrane receptor-dependent agonists CCK-8, ACh, PGE₂, and the receptor-independent agonist KCl induced a dose-dependent contraction of muscle cells from sham-operated and from AC animals. The CCK-8 dose-response relationship in the sham-operated group was significantly higher than that in the AC group (Fig. 1, P < 0.05, by ANOVA); 10 nM CCK-8 caused a maximal shortening of 20.3 ± 1.0% in sham-operated guinea pigs compared with only 13.0 ± 0.2% in AC animals. Similar results were observed with ACh (Fig. 2, P < 0.01, by ANOVA) and KCl (Fig. 3, P < 0.01, by ANOVA) induced muscle cell contraction in these two conditions.
groups. However, the PGE2-induced contraction was unaffected in AC (Fig. 4).

To examine the functional integrity of the signal transduction pathway, muscle cells were stimulated with the G protein activator GTP\(\gamma\)S and s messengers IP3 and DAG. GTP\(\gamma\)S and IP3 were used in muscle cells permeabilized by brief exposure to saponin to allow GTP\(\gamma\)S and IP3 to diffuse across the plasma membrane. DAG was used in intact muscle cells. GTP\(\gamma\)S caused a dose-dependent contraction of muscle cells from both sham-operated and AC guinea pigs with maximal contractions of 20.6 \(\pm\) 0.5 and 20.1 \(\pm\) 0.2\%, respectively (Fig. 5). The magnitude of contraction caused by GTP\(\gamma\)S in these two groups was not different from that induced by CCK-8 in intact cells from sham-operated gallbladders. Similar results were observed after the muscle cells from both groups were treated with IP3 (Fig. 6). There was also no significant difference between sham-operated guinea pigs and those with AC in the DAG-induced contraction (Fig. 7). These findings suggest that with the exception of the PGE2-mediated effects, the muscle defect associated with acute inflammation appears to be localized in the plasma membrane. However, the signal transduction distal to the membrane receptors is unaffected.

To support this hypothesis, CCK receptor binding studies were performed. In the control group, the total binding of CCK receptors (in the absence of unlabeled CCK) was 14.2 \(\pm\) 0.47\% with specific binding of 12.1 \(\pm\) 0.24\% and nonspecific binding of 2.2 \(\pm\) 0.31\% (Fig. 8). Scatchard analysis based on this data showed that the binding capacity of CCK receptors was 0.29 \(\pm\) 0.02 pmol/mg protein. In animals with AC, the total binding of CCK receptors decreased to 6.2 \(\pm\) 0.35\% with a specific binding of 5.9 \(\pm\) 0.13\% and nonspecific binding of 1.1 \(\pm\) 0.29\%. The binding capacity of CCK receptors was 0.16 \(\pm\) 0.05 pmol/mg protein, which was lower than that of control group \((P < 0.01)\). These data suggest that the CCK receptors have been damaged by the inflammatory process.

PGE2 receptor binding studies were also carried out (Fig. 9). In the control group, the total binding of PGE2 receptors (in the absence of unlabeled PGE2) was 7.0 \(\pm\) 0.3\%.
1.45% with a nonspecific binding of 1.7 ± 1.21% and specific binding of 5.8 ± 0.17%. The binding capacity was 0.25 ± 0.02 pmol/mg protein. In animals with AC, the total binding of PGE₂ was 7.2 ± 1.18% with nonspecific binding of 1.5 ± 1.33% and the specific binding of 5.3 ± 0.11%. The binding capacity was 0.21 ± 0.03 pmol/mg protein. These differences were not significant, suggesting that the functional integrity of the PGE₂ receptors be preserved in AC.

To determine the mechanism responsible for the damage of membrane receptors and ion channels during acute inflammation, the constituent of membrane phospholipids were investigated (Fig. 10). Data showed a significant reduction in the phospholipid content with normal cholesterol content in muscle cells from AC. The phospholipid content in sham-operated gallbladders was 1.26 ± 0.04 μmol/mg protein compared with 0.97 ± 0.05 μmol/mg protein in AC (P < 0.01), resulting in a higher cholesterol-to-phospholipid ratio in AC of 0.25 ± 0.02 compared with 0.18 ± 0.02 for controls (P < 0.05). These results suggest that acute inflammation affects the composition of the plasma membrane, especially the phospholipid component that may account for the impaired muscle cell contraction and reduced binding capacity of membrane receptors.

The lower phospholipid content in plasma membranes in AC could be due to over consumption by lipid peroxidation that is frequently associated with acute inflammatory processes. Therefore, lipid peroxidation was determined in gallbladder muscle membranes from sham-operated and AC. The magnitude of lipid peroxidation in sham-operated guinea pigs was 163.6 ± 45.5 nmol of MDA/100 mg membrane protein, which was significantly lower than 527.3 ± 19.8 nmol of MDA/100 mg membrane protein in gallbladder muscle membrane from AC (P < 0.001).

The H₂O₂ content was measured in the muscle layer because acute inflammatory processes induce lipid peroxidation by generating free radicals. The total H₂O₂ content was determined in whole cell homogenate because H₂O₂ can cross the membrane freely. In the control group, H₂O₂ content was 3.13 ± 0.56 μM/mg.
protein, which was significantly lower than 17.6 ± 0.61 μM/mg protein in AC (P < 0.001). These data suggest that free radicals may be responsible for the damage to the plasma membranes.

As previously mentioned, the PGE2-induced muscle contraction and the functional integrity of PGE2 receptors were unaffected in AC. One possible explanation for this unexpected finding is that increased production of the lidgand could protect its receptors from agents that damage or inactivate them (14). The PGE2 content of the ligand could protect its receptors from agents for this unexpected finding is that increased production of free radicals may be responsible for the damage to the plasma membranes.

The activities of free radical scavengers were also measured. PGE2 pretreatment increased significantly in cytosol of muscle cells from AC (P < 0.001). Similar results were seen with SOD activity, which increased from 2.9 ± 2.2 in controls to 16.5 ± 5.2 U/mg protein in AC (P < 0.01). These data suggest that the increase in PGE2 content and preservation of its receptors in AC are cytoprotective responses against the inflammatory process.

To further confirm this hypothesis, catalase and SOD activities were measured in normal muscle cells pretreated in vitro with exogenous PGE2 for 30 min. As shown in Fig. 12, the activities of catalase and SOD increased significantly in cells pretreated with PGE2 to levels similar to those observed in AC. Catalase activity increased from 9.4 ± 2.1 to 25.3 ± 4.7 U/mg protein, and SOD activity increased from 2.9 ± 3.5 to 14.0 ± 2.4 U/mg protein (P < 0.001 and P < 0.01).

**DISCUSSION**

These studies show that acute inflammation in the guinea pig gallbladder affects the muscle contraction. It impairs the response to agonists that act on membrane constituents such as CCK-8 and ACh that stimulate the receptor-independent agonist such as KCl, which contracts muscle cells by increasing calcium influx. These abnormalities are similar to those found in muscle cells from gallbladders with bile saturated with cholesterol (5). However, there are also significant differences, particularly in the contraction induced by PGE2 which remains normal in AC.
The finding that the initial muscle damage in experimental AC is confined to the plasma membrane is also supported by biochemical studies. Measurements of membrane lipids reveal that the phospholipid but not the cholesterol content of the plasma membrane of AC muscle cells is reduced resulting in a higher cholesterol-to-phospholipid ratio. Higher cholesterol-to-phospholipid ratios decrease the fluidity of plasma membranes (5), which could affect the functions of transmembrane proteins and calcium channels (5, 43). These abnormalities could explain the defective muscle cell contraction in response to CCK-8, ACh, and KCl. However, in contrast to the defective contraction induced by PGE$_2$ in gallbladders with cholesterol stones (48), the contraction caused by PGE$_2$ in muscle cells from gallbladders with AC is normal. These findings suggest that mechanisms other than a decrease in membrane fluidity are more likely to cause this impaired contraction because the actions of PGE$_2$ are also mediated by transmembrane receptor (9). The mechanisms whereby PGE$_2$ receptors and their functions are conserved are not fully understood (29, 30, 49). It is conceivable that this receptor preservation is due to the increased PGE$_2$ generation (53).

The reduction in the phospholipid content may be due to an increase in lipid peroxidation in the plasma membranes caused by the inflammation of the muscle cells as demonstrated by the higher production of TBA metabolites. This finding also suggests that the muscle damage may be mediated by reactive oxygen species. These reactive oxygen species could be released by polymorphonuclear cells that heavily infiltrate the gallbladder wall (11, 46) or generated within the muscle cells themselves induced by the presumed chemical factors that induce AC. The finding that reactive oxygen species are significantly increased in muscle cells from specimens affected by AC is consistent with this hypothesis.

The possibility that reactive oxygen species are involved in the pathogenesis of the muscle dysfunction in AC is further supported by the finding that these muscle cells respond to the inflammatory injury with cytoprotective mechanisms that are involved in neutralizing their deleterious effects (23). Many cells, including muscle cells, respond to reactive oxygen species with an increase in prostaglandins, particularly PGE$_2$ and scavengers of reactive oxygen species (15, 18, 26, 28, 36). The PGE$_2$ content of the gallbladder muscle was increased, and the activities of SOD and catalase were also elevated in this model of experimental AC. SOD converts monochloramine into hydrogen peroxide that is then inactivated by catalase (21, 25).

Reactive oxygen species may also damage transmembrane receptors by affecting SH radicals (12). The binding capacity of CCK-A receptors is markedly reduced in muscle cells from specimens with AC. Similar damage may occur to other receptors (cholinergic receptors) and calcium channels with the exception of receptors that can be protected by their ligands such as it seems to occur with PGE$_2$ receptors.

In summary, muscle cells from experimental AC are affected by and respond to acute inflammation. The initial injury appears to be confined to the plasma membranes affected by chemical factors that still need to be elucidated. However, reactive oxygen species may be one of the factors that may cause this muscle dysfunction either by inducing lipid peroxidation and changes in the phospholipid bilayer or by acting directly on SH radicals of transmembrane receptor (12). The relationship between the preservation of the functional integrity of PGE$_2$ receptors and pathways and the increased synthesis of reactive oxygen species scavengers needs further studies.

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