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PYROGENIC AND INFLAMMATORY PROPERTIES OF CERTAIN BILE ACIDS IN MAN *

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This is a report on the pyrogenic and inflammatory properties of certain bile acids in man. The study was prompted by the structural similarity between these acidic steroids and the pyrogenic neutral steroids described previously (2–7). In addition, it seemed important to establish whether the large quantities of steroid acids formed during the metabolism of cholesterol could serve as a source of endogenous compounds having fever-producing action in man.

METHODS AND RESULTS

Nineteen bile acids and derivatives were examined for fever-producing activity after their intramuscular administration to adult volunteer hospital patients. Lithocholic acid was repeatedly recrystallized to insure purity. The glycine, taurine, and acetyl derivatives of lithocholic acid were synthesized and purified in these laboratories; the other compounds were obtained commercially and used without additional purification. All bile acids were dissolved in propylene glycol, in concentrations of 12.5 and 25.0 mg per ml, and because of their acidity these solutions were neutralized with dilute base. The general details of the study, including precautions taken to exclude bacterial pyrogen contamination, were similar to those described in a previous report (3). The compounds examined, together with the incidence of pyrogenic responses which followed their injection, are listed below.

1. Lithocholic acid (3α-hydroxycholanic acid). Nineteen subjects received 22 intramuscular injections of 6 to 50 mg each. Pyrogenic responses at different doses were as follows: 6 mg, 5 of 5; 12 mg, 6 of 7; 25 mg, 7 of 8; 50 mg, 1 of 2. In general, pyrogenic reactions were similar to those previously described with neutral steroid pyrogens and the responses of three subjects to injection of 6 mg of this bile acid are shown in Figure 1. At this small dose, lithocholic acid appeared to be more intensely pyrogenic than either etiocholanolone or pregnanolone (4, 6).

Local inflammatory reactions were regularly observed after injection of lithocholic acid. The onset of inflammation, characterized by the usual physical signs, was variable, ranging from 6 to 30 hours after injection. The inflammation usually increased for 2 to 3 days and then gradually regressed, with an indolent course sometimes lasting 2 to 4 weeks. Histologically, biopsies of injection sites showed edema and necrosis of tissue, with a marked polymorphonuclear infiltrate. Further details will form the basis of a subsequent report. A photomicrograph of a biopsy taken 4 days after injection is shown in Figure 2. It is of interest that the period of most intense inflammation usually occurred well after the fever had subsided.

Constitutional symptoms such as headache, malaise, nausea, anorexia, and so forth, were intense, and patients frequently complained of an unusually strong sense of fatigue. Myalgias and arthralgias were less frequent than those observed after injections of neutral steroid pyrogen.

2. 3-Acetyl lithocholic acid. This compound was administered to four subjects in single doses of 25 mg each. All developed intense pyrogenic reactions, although in one the onset of fever was delayed until the day after injection. Inflammatory reactions and constitutional symptoms were comparable to those produced by lithocholic acid.

3. 24-Methyl lithocholic acid. One subject received 25 mg and two received 12 mg each. All developed intense pyrogenic responses, the larger dose causing a prolonged fever lasting 5 days. Inflammatory reactions and constitutional symptoms were correspondingly severe. Because of the intensity of these responses, no further testing was done.

4. Glycolithocholic acid. Five subjects received 12 mg and four received 25 mg each. In addition, two other subjects received 21 mg each, the steroid solution having been sterilized by Seitz filtration rather than by autoclaving. All subjects, except one, developed pyrogenic and inflammatory reactions similar to those obtained with unconjugated lithocholic acid. The single subject who did not develop a fever after receiving an injection of 12 mg, did develop a 2×2 cm area of inflammation at the site of injection. Chromatographic analysis of the glycolithocholic acid used in these injections showed it to be free of unconjugated precursor.

5. Taurolithocholic acid. Six subjects received 12 mg and four received 25 mg each. The maximum temperature reached in the group receiving 12 mg was 38.3° C

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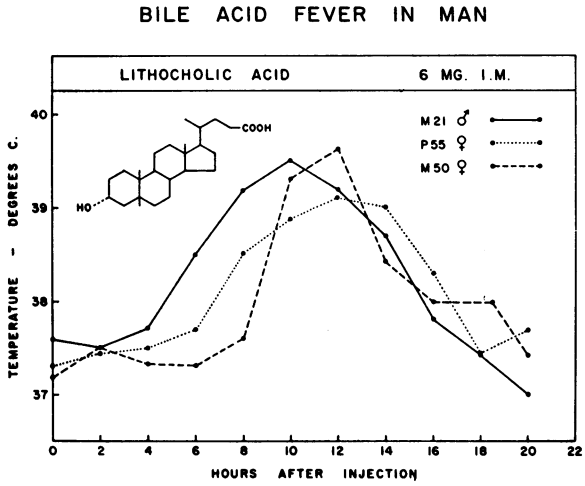

FIG. 1. FEBRILE RESPONSES OF THREE SUBJECTS TO THE INTRAMUSCULAR INJECTION OF 6 MG OF LITHOCHOLIC ACID.

(one subject), and in the group receiving 25 mg, 38° C (one subject). All other patients remained afebrile despite the presence in all of intense local inflammatory reactions which were frequently as severe as those produced by glycolithocholic or lithocholic acids. Because of the severity of these reactions, no attempt was made to elicit fever with doses greater than 25 mg. Figure 3 shows a biopsy of one reaction to an injection of 25 mg together with the corresponding temperature record.

In addition to the consistent development of intense inflammatory reactions, unaccompanied by fever, most subjects receiving taurolithocholic acid complained of marked constitutional symptoms, especially anorexia and a strong sense of fatigue.

6. Hyodeoxycholic acid (3α,6α-dihydroxycholanic acid). Five subjects received 100 mg each. One developed a marked febrile response, two developed low grade but significant fevers, and two subjects had no febrile responses.

7. Ursodeoxycholic acid (3α,7β-dihydroxycholanic acid). This compound was tested in nine subjects in amounts of 100 mg each. One subject developed a moderately intense fever, three responded with small but significant temperature rises, and five showed no temperature elevations.

Despite several pyrogenic reactions among the subjects receiving these last two compounds, no inflammatory reactions were noted grossly and none of the subjects complained of local tenderness. However, three subjects (two of whom remained afebrile) complained of some of the previously described constitutional symptoms after injection of ursodeoxycholic acid.

8. The following twelve compounds were nonpyrogenic when tested in at least six subjects in doses of 100 mg each: 7-ketolithocholic acid (3α-hydroxy-7-ketocholanic acid); 3,6 diketocholanic acid; chenodeoxycholic acid (3α,7α-dihydroxycholanic acid); deoxycholic acid (3α,12α-dihydroxycholanic acid); apocholic acid (3α,12α-dihydroxy-Δ8-14-cholenic acid); diketolithocholic acid (3α-hydroxy-7,12-diketocholanic acid); dehydrocholic acid (3,7,-12-triketocholanic acid); cholic acid (3α,7α,12α-trihy-

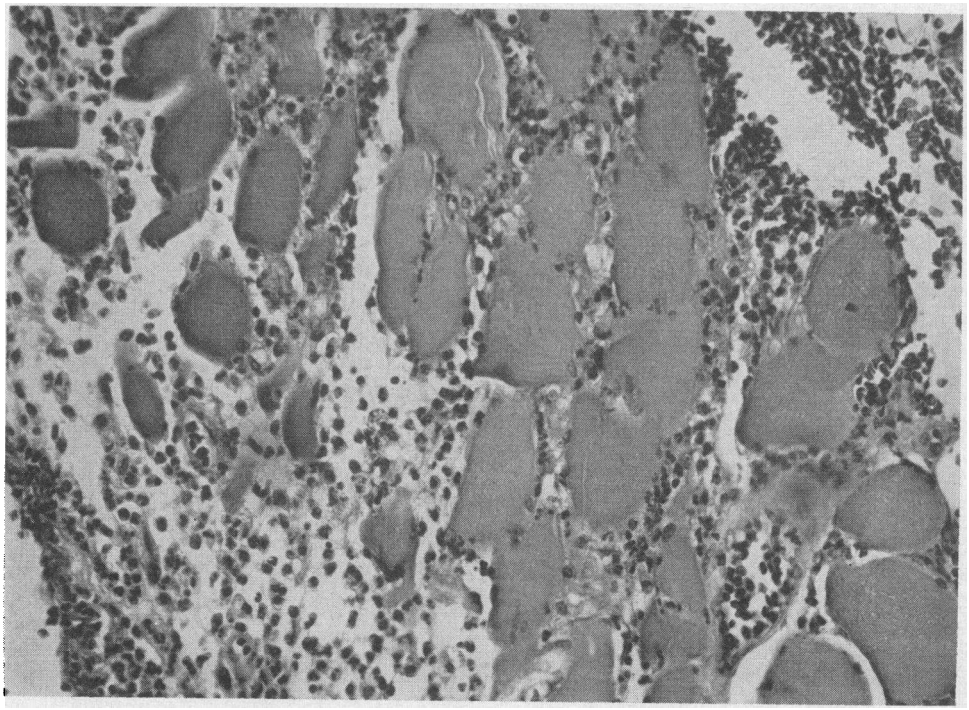

FIG. 2. ACUTE INFLAMMATORY REACTION AFTER THE INTRAMUSCULAR INJECTION OF LITHOCOLIC ACID. (× 245)

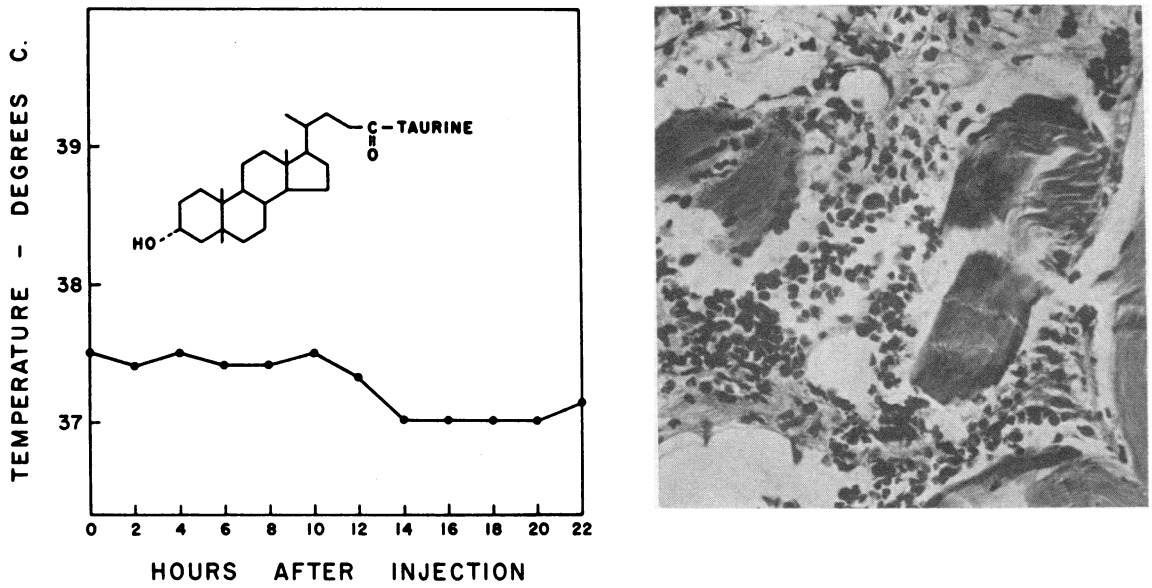

FIG. 3. ACUTE INFLAMMATORY REACTION AFTER THE INTRAMUSCULAR INJECTION OF 25 MG OF TAUROLITHOCHOLIC ACID IN THE ABSENCE OF ANY FEBRILE RESPONSE. (× 290)

droxycholanic acid); glycocholic acid; taurocholic acid; and lithobilianic acid.

Among these patients there were scattered instances of mild temperature elevations (or absence of normal nocturnal temperature depressions), but these did not form a consistent pattern and were not considered significant. In addition, no inflammatory reactions or constitutional symptoms developed after injection of these steroids.

DISCUSSION

The pyrogenic neutral steroid hormone metabolites described previously belong to the 5β-H series of compounds, in which the hydrogen atom projects in front of the plane of the nucleus, and the A : B ring junction is angular (*cis*). This molecular configuration also characterizes the bile acids, which represent the principal steroid end products of cholesterol metabolism. The large amounts of these steroid acids formed daily from the breakdown of cholesterol thus represent a large potential source of endogenous fever-producing agents in man.

The present investigations demonstrate that several of these biliary compounds do indeed have powerful thermogenic and inflammatory properties when administered intramuscularly to humans. Thus they extend the number of known steroid pyrogens to include nonhormonal derivatives and stimulate interest in the possible participation of these toxic biliary substances in clinical disorders in a manner previously demonstrated for the neutral steroid pyrogen, etiocholanolone (8, 9). The activity of lithocholic acid and its conjugates demonstrated in this study makes these monohydroxy compounds of special interest in this regard, although these substances may prove to be only the most potent of a series of pharmacologically active related steroids.

The origin of lithocholic acid and its derivatives and their concentrations in tissues, fluids, and intestinal contents are not well known. However, approximately 1,000 mg of cholesterol is degraded to bile acids daily (10, 11). During this process, hydroxylation of the nucleus occurs, first at carbon 7 and then at carbon 12, resulting in the two principal bile acids in man, chenodeoxycholic and cholic acids. Lithocholic acid could be formed either by failure of C-7 hydroxylation initially or through subsequent bacterial dehydroxylation in the intestine. In the formation of bile acids, oxidation of the terminal side chain seems to prevent further nuclear hydroxylation, and the timing of this process is thought to determine the ratio of dihydroxy to trihydroxy bile acids. Presumably, thyroxine increases the chenodeoxycholic acid: cholic acid ratio in bile by stimulating this oxidation (11, 12). Similarly, oxidation of the side chain prior to C-7 hydroxylation could result in an increased production of the monohydroxy

derivative, lithocholic acid. Carbon 7 dehydroxylation of cholic acid by intestinal bacteria is the major source of deoxycholic acid (11), and an analogous process could result in the formation of lithocholic acid from chenodeoxycholic acid. In any event, lithocholic acid has been isolated from the bile and feces of normal subjects in significant amounts (13–16), and alterations in the degradative pathway of cholesterol could lead to excessive production of this highly toxic metabolite. Although there is no present evidence of the participation of this pyrogenic and inflammatory steroid in clinical disease, it is of interest that this compound can produce experimental cirrhosis of the liver in other species (17–19).

Suppression of the thermogenic and inflammatory properties of bile acids by polyhydroxylation of the nucleus, as observed in this study, is consistent with the generalization that additional chemical functions which project from the rear surface of the molecule inhibit these activities. This type of interference has been postulated for enzyme-substrate reactions in other steroids (20–23). Hence, α-oriented oxygen functions at carbons 7 and 12 in the bile acids and at carbon 17 in the neutral steroids inhibit pyrogenicity. In contrast, β-orientation of the carbon 11 oxygen substituent in neutral steroids has little effect on fever-producing activity (4). The two dihydroxy bile acids found to have weak and irregular thermogenic action (ursodeoxycholic and hyodeoxycholic acids) have additional hydroxyl groups in the 7β and 6α positions, respectively, and although the equatorial orientation of both hydroxyls may account for incomplete suppression of fever-producing action, individual differences in response to injected steroids, noted previously (6), may be of some importance in determining the consistency of the pyrogenic reaction to these compounds.

The powerful thermogenic and inflammatory properties of chemical and physiologic conjugates of lithocholic acid are of some interest and potential significance. It has generally been assumed that *in vivo* conjugation of steroids represents in part an inactivating process, and previous studies with neutral steroid pyrogens demonstrated that chemical esters such as etiocholanolone and pregnanolone acetates, as well as physiologic conjugates such as the sulfate and glucosiduronate derivatives of etiocholanolone, were devoid of fever-producing activity (24, 25). Bile acid pyrogens appear to differ strikingly from the neutral steroids in this regard. Esterification of lithocholic acid at both terminal positions of the steroid nucleus, exemplified by 3-acetyl lithocholic and 24-methyl lithocholic acids, did not result in suppression of pyrogenicity. Indeed the latter compound produced both prolonged and intense fever. Moreover, both physiologic conjugates of this bile acid retained powerful thermogenic or inflammatory activity. Thus it would appear that conjugation processes do not necessarily terminate the biological activity of steroid pyrogens, a consideration which must be taken into account when evaluating the possible role of these steroids in the pathogenesis of certain clinical disorders (1, 7).

It is also apparent that the nature of the conjugating substance may determine the type of pharmacologic action manifested by certain steroids, since in the present study dissociation of the inflammatory from the pyrogenic action of lithocholic acid was determined by the type of conjugating amino acid. Thus, alterations in the ratio of taurine : glycine conjugates, which are known to occur with changes in age, diet, or in liver disease (26–28), may alter the possible biological effects of endogenously produced lithocholic acid.

The ability of taurolithocholic acid to produce intense local inflammatory reactions as well as constitutional symptoms, *without significant fever,* is of special importance and implies that these effects may be independent pharmacologic properties of bile acids. Thus the production of constitutional symptoms may not be directly the result of fever, nor does *nonspecific* inflammation per se explain the mechanism of steroid-induced fever in man.

SUMMARY

Nineteen free and conjugated bile acids were examined for pyrogenic properties. The results of this study indicate that:

1. The endogenous biliary steroid, lithocholic acid, has significant inflammatory and pyrogenic action in man. It is possible that aberrations in the degradation of cholesterol to bile acids or enteric microbial dehydroxylation of these compounds might result in excessive production of this extremely active steroid pyrogen which, by in-

ference, could then participate in febrile and inflammatory clinical disorders.

2. The potent pharmacologic properties of chemical and physiologic esters of lithocholic acid demonstrate that *in vivo* conjugation processes do not necessarily terminate the biological activity of steroids.

3. Production of intense inflammation without significant fever by taurine-conjugated lithocholic acid strongly suggests that nonspecific inflammation per se does not explain the mechanism of steroid fever in man.

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