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 (3a-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 x 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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Bile Acid Fever in Man

**Fig. 1.** Febrile Responses of Three Subjects to the Intramuscular Injection of 6 mg of Lithocholic Acid.

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-dihydroxy-Δ8-14-cholenic acid); apocholic acid (3α,12α-dihydroxy-Δ8-Δ14-cholenic acid); diketolithocholic acid (3α-hydroxy-7,12-diketocholanic acid); dehydrocholic acid (3,7,12-triketocholanic acid); cholic acid (3α,7α,12α-trihy-
droxycholanic acid; glycocholic acid; taurocholic acid; and lithobilanic 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 dehydroxy-
lation 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 degra-
dative pathway of cholesterol could lead to ex-
cessive production of this highly toxic metabolite.
Although there is no present evidence of the par-
ticipation of this pyrogenic and inflammatory ster-
oid 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 inflam-
matory properties of bile acids by polyhydroxylation
of the nucleus, as observed in this study, is con-
sistent 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 ca-
rbons 7 and 12 in the bile acids and at ca-
rbon 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 thermo-
genic action (ursodeoxycholic and hydoxycho-
litic acids) have additional hydroxyl groups in the
7β and 6α positions, respectively, and although the
equatorial orientation of both hydroxyls may ac-
count for incomplete suppression of fever-produc-
ing 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 po-
tential significance. It has generally been as-
sumed that in vivo conjugation of steroids repre-
sents in part an inactivating process, and previous
studies with neutral steroid pyrogens demon-
strated that chemical esters such as etiocholanolone
and pregnanolone acetates, as well as physiologic
conjugates such as the sulfate and glucosidu-
rone derivatives of etiocholanolone, were devoid
of fever-producing activity (24, 25). Bile acid
pyrogens appear to differ strikingly from the neu-
eutral steroids in this regard. Esterification of litho-
cholic acid at both terminal positions of the steroid
nucleus, exemplified by 3-acetyl lithocholic and
24-methyl lithocholic acids, did not result in sup-
pression of pyrogenicity. Indeed the latter com-
pound produced both prolonged and intense fe-
ver. 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 con-
sideration 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 con-
jugating substance may determine the type of
pharmacologic action manifested by certain ster-
oids, since in the present study dissociation of the
inflammatory from the pyrogenic action of litho-
cholic acid was determined by the type of conju-
gating amino acid. Thus, alterations in the ra-
tio of taurine : glycine conjugates, which are known
to occur with changes in age, diet, or in liver dis-
ease (26–28), may alter the possible biological ef-
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 pro-
erties of bile acids. Thus the production of con-
stitutional symptoms may not be directly the re-
sult 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 en-
teric microbial dehydroxylation of these com-
pounds 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.

REFERENCES


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