D-600 Sensitive and Insensitive Spontaneous Contractions in the Guinea-Pig Hepatic Duct

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Suzuki, H., Fukushi, Y. and Wakui, M. D-600 Sensitive and Insensitive Spontaneous Contractions in the Guinea-Pig Hepatic Duct. Tohoku J. exp. Med., 1988, 156 (1), 13-21 — The mechanical properties of the longitudinally cut preparations of the guinea-pig hepatic duct were studied. About 42% of the preparations (18 of 43 preparations) showed spontaneous phasic contractions which were unaffected by either tetrodotoxin, guanethidine or atropine. About 60% of them exhibited phasic contractions which appeared at irregular frequencies in the same tissue and varied in size. This sort of contractions usually accompanied small contractions. In about 20%, contractions occurred constantly in frequency (about 6-14 every 10 min) and in size. The remaining 20% showed twitch-like contractions which occurred sporadically. These contractions were classified into two types depending upon the susceptibility to the Ca²⁺ entry blocker, D-600. D-600 (1-100 μM) strongly suppressed both regularly occurring contractions and twitch-like ones. On the other hand, the drug was ineffective on irregularly occurring contractions. The Ca²⁺ sources underlying these contractions were discussed. ——— hepatic duct; spontaneous contractions; D-600; guinea-pig

Secreted from the liver cells, the bile is transported eventually into the intestinal cannal through the hepatic, cystic ducts, gall bladder, common bile duct and sphincter of Oddi. As far as the mechanism of bile transport in these organs except the hepatic duct, there have been many reports suggesting that the contractile ability of smooth muscles constituting the wall of the passage is essential; cystic duct; (Mroczka et al. 1982), gall bladder; (Bainbridge and Dale 1906; Okada 1915; McMaster and Elman 1926; Ivy 1934; Ludwick and Bass 1967; Itoh and Takahashi 1981; Itoh et al. 1982), common bile duct; (Ludwick and Bass 1967), sphincter of Oddi; (Behar and Biancani 1980, 1982; Hogan et al. 1982). On the other hand, the mechanism of bile transport in the hepatic duct has been little understood, probably for the reason of difficulty in obtaining the isolated preparations. Morphological observations have shown that the hepatic ducts in human being is formed by dense fibrous tissue which contains many
elastic fibers (Stahle 1952). This suggests that the hepatic ducts may be composed of smooth muscle cells as in the cases of other biliary ducts. However, a study of the membrane potential of the smooth muscle of the hepatic duct using the microelectrode technique has suggested no active contractions in this organ, because the measured membrane potential was quite stable (Creed and Kuriyama 1971). Very recently, however, the presence of contraction was demonstrated under the microscope even in canaliculi consisted by doublet liver cells (Phillips et al. 1982; Watanabe and Phillips 1986).

The purpose of the present study, therefore, is to examine possible contractile activity in the hepatic duct with isometric tension recording.

**MATERIALS AND METHODS**

Hepatic ducts were excised from male guinea-pigs weighing 200-300 g which were sacrificed by a blow on the head. Both cystic and common bile ducts were carefully tied by thread respectively to avoid possible scatter of bile content. Hepatic ducts were then isolated together with cystic and common bile ducts. They were cleaned from connective tissues and liver cells surrounding smooth muscles. Hepatic ducts were opened by a longitudinal cut. This provided a longitudinal smooth muscle preparation of about 5 mm in length and 3 mm in width. The preparation was mounted in a 1 ml organ bath containing a standard physiological salt solution of the following compositions (mM): NaCl 117.2, KCl 5.9, CaCl$_2$ 2.0, MgCl$_2$ 1.2, NaH$_2$PO$_4$ 1.2, NaHCO$_3$ 25.0, glucose 11.0. The solution was maintained at 30°C and bubbled with a mixture of 95% O$_2$ and 5% CO$_2$. Resting tension was adjusted at about 40 mg and isometric tension was recorded with a force transducer (Shinkoh U gage, 10 g, Nagano). To avoid any loss of magnitude of contraction, the muscle was connected at a distance of not more than 10 mm from the loci of the force transducer. Before the start of experiments, all preparations were allowed to equilibrate for several hours. In a Ca free solution, 1 mM EGTA was added, and the concentration of MgCl$_2$ was elevated to 6.2 mM for the membrane stabilization (Bulbring and Tomita 1970). When the effects of D-600 were examined, 10 μl of D-600 solution at an appropriate concentration was added directly to the bath to give desired concentrations. Concentration-response curves for D-600 were constructed by increasing its concentrations from 0.01 μM. When the data were statistically analysed, each one of constant responses was compared before and after the treatment with the drug. All data in the text are expressed as mean ± s.e. D-600 was kindly supplied from Eisai Co., Ltd. (Tokyo).

**Results**

When the preparations of hepatic ducts were allowed in physiological solution, 18 of 43 preparations (about 42%) showed spontaneous phasic contractions in the longitudinal direction and the remaining preparations were quiescent. In most of preparations which showed spontaneous contractions, the contractions occurred several ten minutes after immersion of tissues into the bath. Neither tetrodotoxin (10 nM-1 μM), guanethidine (1 μM) nor atropine (0.1-1 μM) had any clear effects on these contractions. As shown in Fig. 1a, in 60% of spontaneously active preparations, contractions appeared irregularly in frequency. The size of each contraction was also not constant. This type of phasic contractions was generally large in magnitude. Roughly measured frequency of the contraction
Contractions in the Hepatic Ducts

Fig. 1. One type of spontaneous contractions observed in the isolated smooth muscle preparation of the guinea-pig hepatic duct and the effects of D-600 on the contractions. (a) Tracings were obtained from the same preparation before (control) and 1 min after D-600 application at the concentrations indicated. The drug was cumulatively added into the organ bath. (b) Concentration-response relation for D-600. The magnitude of each phasic contraction (indicated by 1, 2 and 3, for example) was measured at the distance from the basal tone (indicated by the dotted line) to the peak of each contraction (indicated by the arrow). The mean of magnitudes of all contractions observed for 10 min was taken as the mean of one preparation. The magnitudes of the contraction before application of D-600 were taken as controls. Each point represents the mean of 10 preparations from different guinea-pigs and vertical bars show s.E. of the mean.

was in the range of 7-9 every 10 min. This type of contractions very often accompanied small contractions occurring at frequencies of about 3-4/min. In order to look into some nature of these contractions in relation to Ca$^{2+}$, the effects of D-600, a Ca$^{2+}$ entry blocker, were further studied. As shown, there were little changes in size or frequency of either large or small phasic contractions by
Fig. 2. The other type of spontaneous contractions observed in the hepatic duct and the effect of D-600 on the contractions. (a) Tracings were obtained before (control) and 1 min after D-600 application at the concentrations indicated, from the same preparation. (b) Concentration-response relation for D-600. The magnitudes of the contraction before application of D-600 were taken as controls. Each point represents the mean of 4 preparations from different guinea-pigs and vertical bars show s.e. of the mean.

application of D-600 at any concentrations (1-100 µM). Fig. 1b quantifies the results of 10 experiments on the large phasic contraction.

Fig. 2a shows another type of spontaneous contractions observed in other 20% of preparations (see control response). Contractions occurred regularly. However, the frequency varied in the range of 6-14 every 10 min, depending upon preparations. The size of contractions was nearly constant in most preparations showing this type of contractions. Small contractions with high frequency seen in preparations showing irregular type of contraction occurred only around the peak of the phasic contractions. Both contractions are not changed by 0.01 µM D-600 in either the magnitude or frequency. However, at higher concentrations
Fig. 3. The effects of D-600 (1 μM) on the twitch-like contractions of the guinea-pig hepatic duct. TEA at 10 mM was added throughout the experiment. The tracing is representative of the responses of 4 preparations from different guinea-pigs.

(0.1-100 μM) D-600 clearly reduced the size of contractions, although little affected the frequency of contraction when observable. Fig. 2b quantifies the results of four experiments.

Fig. 3 shows twitch-like contractions. This type of contractions was observed in about 20% of preparations which exhibited spontaneous contraction. The size of contraction of this type was nearly constant. This type of contraction sometimes appeared in the preparation with one of former types of contractions. When tetraethylammonium (TEA) was added into the bath, the magnitude of the contraction became larger and the frequency became higher and constant. The drug produced twitch-like contractions also in some of the quiescent preparations. About 3 min after the addition of D-600 at 1 μM to the bath, the contractions were completely suppressed. In order to obtain more information with Ca²⁺ mechanism of spontaneous contraction, the muscle with spontaneous contractions was perfused with zero Ca and 1 mM EGTA containing solution. In this Ca²⁺-free solution, the twitch-like contractions rapidly ceased, but not the irregular type of contractions even about 40 min after the perfusion (Fig. 4).

Fig. 4. The effects of Cal-removal on the spontaneous contractions of the guinea-pig hepatic duct. Ca²⁺-free, 1 mM EGTA containing solution was perfused at a flow rate of about 1 ml/min at the arrow indicated.
DISCUSSION

One of interesting findings in the present study is that some of the guinea-pig hepatic duct exhibit spontaneous contractions. Since these contractions are insensitive to TTX, guanethidine or atropine, they may be myogenic in origin.

It has been generally believed that bile is secreted at a pressure of 15-25 cm H$_2$O and ceases when the pressure in the common bile duct rises to 35 cm H$_2$O. The sphincter of Oddi offers a resistance to bile flow of 10-25 cm H$_2$O (Scherlock 1981). In that case, what can be the driving force of bile movement at least into the common bile duct?

The first possible force is a hydrodynamic pressure in the space downstream from bile canaliculus. There, the hydrodynamic pressure must increase as a bile volume increasing with an elevation of osmorality in the bile due to secretion of ions and other substances from liver cells. This manner of bile movement at least in the hepatic duct has been proposed by Creed and Kuriyama (1971) from their findings with microelectrode technique that in smooth muscles of the guinea-pig hepatic duct, there was no spontaneous change in the membrane potential. In the present experiments, about 60% preparations did not show spontaneous contractions. This fact may also support the above hypothesis. In addition, spontaneous phasic contractions in the remaining 40% preparations might augment hydrodynamic pressure efficiently to drive bile flow to common bile duct.

The second possible force driving bile into the hepatic duct is a contractile property of canaliculus. It was recently shown that the canaliculus could be contracted by an increase in intracellular Ca$^{2+}$ concentration of liver cells (Phillips et al. 1982; Watanabe and Phillips 1986). Although it is not clear at present if the contraction of canaliculus is sufficient for moving all the bile into the hepatic duct, this force must be playing at least a part of it.

As the third possible force for the bile movement in the hepatic duct, we can show here a contractility of smooth muscle of the hepatic duct. In the present studies we recorded spontaneous contractions of the hepatic duct only in the longitudinal direction. However, at least in the alimentary cannal, it is well known that a contraction in longitudinal smooth muscles happens together with that in electrically connecting circular smooth muscles (Conner et al. 1977). Therefore, the repeated phasic contractions observed in the hepatic duct should produce a peristalsis in this organ. This seems to give a strong force for the bile transport as is the case of alimentary transport in the gut.

The other interesting finding in the present study is that these spontaneous contractions can be classified into two types depending upon the susceptibility to the Ca$^{2+}$ blocker, D-600, which is well known to possess a high specificity in blocking voltage-dependent Ca$^{2+}$ channels (Golenhofen and Lammel 1972; Haeusler 1972; Mayer et al. 1972; Riemer et al. 1974; Nawrath et al. 1977) and therefore Ca$^{2+}$-action potential dependent contractions. One type of phasic
Contractions in the Hepatic Ducts 19

contractions was sensitive to D-600, suggesting that the Ca\(^{2+}\) source for this type is outside of the membrane. In this type, there were two subtypes classified by difference in time course of contraction and its appearance manner. In one subtype, the contraction was long lasting (about 1 min from the start to the end), and appeared repeatedly at nearly regular frequency. This contraction is thought to be caused by spontaneous periodic action potential firing based on some pacemaker potentials as in the case of some other smooth muscles (guinea-pig portal vein and guinea-pig taenia coli; Golenhofen and Lammel 1972; Haeusler 1972; Riemer et al. 1974, rat uterus; Reiner and Marshall 1975, guinea-pig gastric antrum; Boev et al. 1976).

In the other subtype, D-600 sensitive contraction was twitch-like in its shape. TEA enhanced this type of contraction in amplitude and appearance of frequency, as in the cases of vascular smooth muscles (rabbit ear artery; Droogmans and Casteels 1976; Droogmans et al. 1977; rabbit pulmonary artery; Haeusler et al. 1980; guinea-pig mesenteric artery; Itoh et al. 1981). Since TEA produced twitch-like contractions in some of the quiescent preparations in the present study (data not shown), it can not be ruled out that in these preparations depolarization may not be large enough to induce muscle contraction.

On the other hand, another type of phasic contractions in the hepatic duct was D-600 insensitive and was not abolished even in a Ca\(^{2+}\)-free, 1 mM EGTA containing solution for 40 min (Fig. 4). This contraction seems to appear without action potentials. In many kinds of smooth muscle cells contraction developed by effective agonists without action potentials (Review; Bolton 1979, vascular smooth muscle, general; Somlyo and Somlyo 1968, guinea-pig stomach; Golenhofen and Wegner 1975; Itoh et al. 1982; guinea-pig mesenteric artery; Itoh et al. 1981; porcine coronary artery: Itoh et al. 1982). As far as a Ca\(^{2+}\) source for such Ca\(^{2+}\)-blocker insensitive contraction, intracellular store is widely acceptable, where Ca\(^{2+}\) can be released through activation of functional receptors on the outer surface of membrane, although in the cat intestinal muscle Ca\(^{2+}\) can release from an intracellular pool not by an agonist but by depolarization of the plasma membrane (Mangel et al. 1982). In the present studies, however, this contraction appeared spontaneously. Therefore, in this tissue Ca\(^{2+}\)-release and ensuring Ca\(^{2+}\)-uptake at the intracellular store may be caused by some unknown signal happens intracellularly. This may result in a small unitary contraction, and possible summation of it may further result in a larger phasic contraction.

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References


Contractions in the Hepatic Ducts


