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Antihistaminic Activity of Lidocaine Derivatives in the Isolated Guinea Pig Ileum

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Local anesthetics (LAs) are drugs that inhibit membrane depolarization by reducing sodium conductance. In addition to the most prominent and well-defined function of LAs described above, numerous studies have shown that LAs possess many other properties, such as anti-inflammatory, anti-arrhythmic or analgesic activities. Furthermore, LAs are structurally similar to histamine H-receptor antagonists. Thus, LAs can be considered antihistaminic drugs. The aim of the present study was to determine and compare the antihistaminic effects of lidocaine derivatives - JCB-1, JCB-2 and JCB-3 - on the isolated guinea pig ileum. Only JCB-1 and JCB-2 at the highest concentration (10^{-4}M) showed the antihistaminic effect. The studied compounds showed a dose-dependent effect. Interestingly, lower concentrations of the studied compounds (10^{-8}M and 10^{-10}M) caused the opposite effect – increased the contraction of the ileum induced by histamine. These effects necessitate further studies. Nevertheless, this is the first time that JCB compounds have been demonstrated to possess antihistaminic properties.

Key words: lidocaine derivatives, guinea pig ileum, antihistaminic activity, histamine

INTRODUCTION

Local anaesthetic agents (LAs) can be defined as drugs which are used to produce reversible interruption of pain impulses in a specific region of the body by direct interaction with voltage-gated Na channels, without loss of consciousness in patients. Most of the LAs consist of an aromatic ring linked by a carbonyl containing moiety through a carbon chain to a substituted amino group. As mentioned previously, LAs cause reversible interruption of the conduction of impulses in peripheral nerves. The primary electrophysiological effect of these drugs is a local decrease in the rate and degree of depolarisation of the nerve membrane, so the threshold potential for transmission is not

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reached and the electrical impulse is not propagated down the nerve. This effect is due to blockade of sodium channels, thereby impairing sodium ion flux across the membrane. In addition to the most prominent and well-defined function of LAs described above, numerous studies have shown that LAs possess many other properties, such as anti-inflammatory, anti-arrhythmic, analgesic, anticonvulsant, antifungal and antibacterial activities (Aydin et al., 2001; Casutto et al., 2006; De Iulis et al., 2001; Frey, 2001; Goodman et al., 2002; Hellström et al., 1988; Hollmann and Durieux, 2000; Pina-Vaz et al., 2000).

The structure of LAs resembles the structure of histamine H-receptor antagonists, suggesting that LAs may influence histamine release. Several studies have shown that lidocaine (LID) used at a concentration lower than that used in infiltration anesthesia inhibits histamine release from mast cells (Kazimierczak et al., 1976). Moreover, some studies had shown that low concentrations of LID or mepivacaine inhibited histamine release from activated mast cells. The effect was more intense when pH was more alkaline (Suzuki et al., 2000). Recent studies suggest that both inhaled and intravenous LID inhibit bronchial contraction caused by histamine (Groben et al., 2007). On the other hand, a few studies have demonstrated that LID may cause bronchial contraction induced by histamine (Hirota et al., 1999).

Even though there are many available LAs, their activity time is not satisfactorily long and they often produce untoward side effects. Hence, the search for a long-lasting, safer LA is the goal of many research studies. The aim of the present study was to determine and compare the antihistaminic effects of LID derivatives - JCB-1, JCB-2 and JCB-3 - on the isolated guinea pig ileum. LID was used as the reference drug.

MATERIALS AND METHODS

Animals

Male and female guinea pigs (300–450 g) were used in the experiment. The animals were housed and fed in a laboratory maintained at constant temperature of 22°C under standard conditions (12:12 h light-dark cycle, standard pellet diet, water *ad libitum*). The treatment of laboratory animals in the present study was in accordance with the respective Polish and European regulations and was approved by the Local Ethics Committee.

Drugs

JCB-1, JCB-2 and JCB-3, LID (*lignocainum hydrochloricum*, Polfa, Poland) were dissolved in distilled water.

Isolated guinea pig ileum

Male and female guinea pigs were sacrificed by cervical dislocation. The ileum was immediately removed and cut into approximately 2.5-cm segments. The segments were placed in a carbogen-aerated organ bath (37°C) containing buffer (120 μM NaCl, 5.6 μM KCl, 2.2 M MgCl, 2.4 M CaCl, 19 μM $NaHCO_3$ and 10 μM glucose). One end of the ileal segment was attached to the transducer (FDT10-A), and the resting tension was adjusted to a load of 0.5 g. All the samples were allowed to equilibrate for at least 30 min before contractions were measured. After 30-minute incubation, 10^{-7} M histamine was added to the buffer and the contraction of the ileum was recorded. Next, the ileum was washed twice with buffer and the compound under investigation was added to the buffer for 15 min. Finally, another concentration of 10^{-7}M histamine was added and the change in ileum contraction strength was recorded. The contractions were recorded using computer software (MP35 Data Acquisition Unit).

Statistical analysis

The data are expressed as mean ± SEM. Differences between treatments and controls were examined for statistical significance using a paired t-test, where the p-value of less than 0.05 indicated a statistically significant difference.

RESULTS

JCB-1 used at 10^{-6}M, 10^{-8}M and 10^{-10}M concentrations increased the strength of ileum contraction by 7.36%, 23.67% and 45.67%, respectively. In contrast, at a concentration of 10^{-4}M the strength

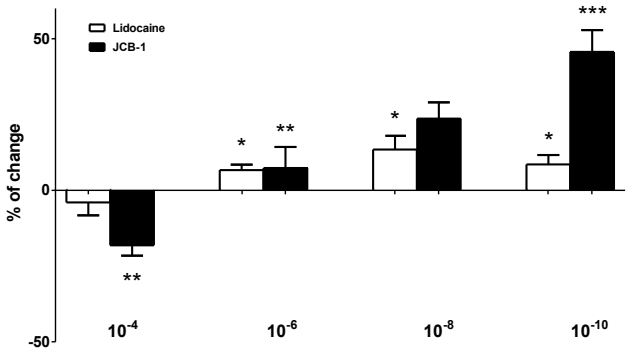

Fig. 1. The effect of JCB-1 and lidocaine on histamine-induced contraction in the guinea pig ileum. The results are expressed as mean ± SEM of 8 samples per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

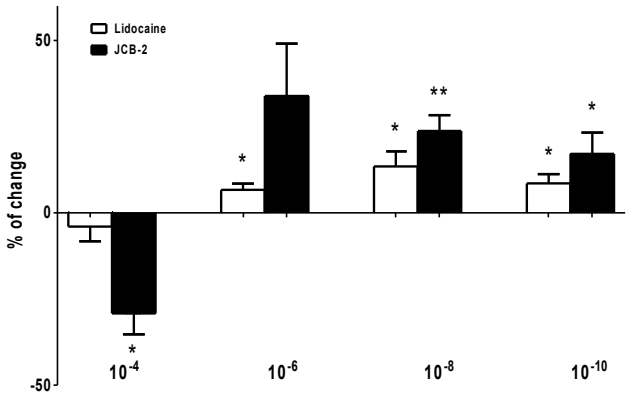

Fig. 2. The effect of JCB-2 and lidocaine on histamine-induced contraction in the guinea pig ileum. The results are expressed as mean ± SEM of 8 samples per group. * $p < 0.05$, ** $p < 0.01$

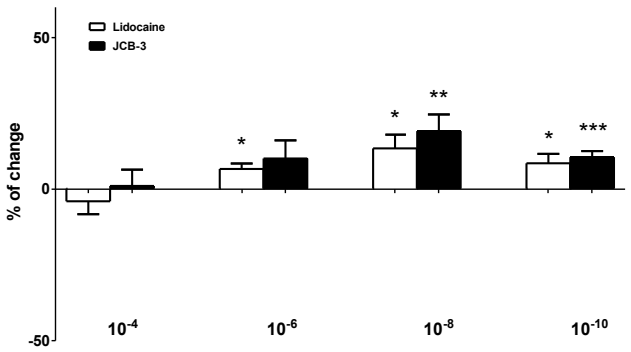

Fig. 3. The effect of JCB-3 and lidocaine on histamine-induced contraction in the guinea pig ileum. The results are expressed as mean ± SEM of 8 samples per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

of ileum contraction was significantly decreased by 18.16%.

Similarly, JCB-2 at concentrations of 10^{-6}M, 10^{-8}M and 10^{-10}M increased the strength of ileum contraction by 33.9%, 23.64% and 17.09%, respectively. However, when used at a concentration of 10^{-4}M, it significantly decreased the strength of ileum contraction by 26.08%. The compound JCB-3 significantly increased the histamine-induced contractions of the guinea pig ileum at concentrations of 10^{-8}M and 10^{-10}M by 19.18% and 10.33%, respectively.

LID at concentrations of 10^{-6}M, 10^{-8}M and 10^{-10}M increased the strength of ileum contractions induced by histamine by 6.66%, 13.47% and 8.55%, respectively. No statistically significant effect was observed at a concentration of 10^{-4}M.

DISCUSSION

The guinea pig ileum is a very common model used to determine whether a potential drug has an antihistaminic effect or not (Larsson et al., 2007). As mentioned previously, the structure of LAs resembles the structure of histamine H_1-receptor antagonists. As the studied compounds are LID derivatives, we attempted to determine their effect on H_1 receptor. Recent data indicate that LAs, except of their local-anaesthetic action, can also have antihistaminic properties (Kazimierczak et al., 1976; Librowski, 2005). It has also been proved that antihistaminic agents exhibit analgesic and anti-inflammatory activities (Panaszek et al., 2007; Tamaddonfard et al., 2008). Furthermore, studies in the guinea pig ileum demonstrate that LAs behave similarly to histamine H_1-receptor antagonists (Horio et al., 1997; Librowski et al., 2009). The studied compounds showed a dose-dependent effect. Only JCB-1 and JCB-2 at the highest concentration (10^{-4}M) showed antihistaminic effect. The effect was much more intense than the effect caused by LID at the same concentration. Considering all the studied compounds, JCB-2 was characterized by the greatest strength; it inhibited ileum contractions induced by histamine at a concentration of 10^{-4}M by 26%. Interestingly, lower concentrations of the studied compounds (10^{-8}M and 10^{-10}M) caused the opposite effect. They increased the contractions of the guinea pig ileum induced by histamine. JCB-1 showed the strongest effect; at a concentration of

10^{-10}M it increased the ileum contraction by 45%. Moreover, the lower the concentration of JCB-1 was, the stronger the effect was observed. Both the increases and the decreases in ileum contractions were much greater for the studied compounds than for LID.

Recent data indicate that LID can either enhance (Hirota et al., 1999; Hirota et al., 2001) or decrease the strength of bronchi or trachea contraction induced by histamine (Downes and Loehning, 1977; Groeben and Peters, 2007). Our results are in agreement with these findings. Perhaps the fact that JCB-1, JCB-2 and JCB-3 are derivatives of LID is associated with their differential influence on contractions of the guinea pig ileum induced by histamine.

The mechanism by which JCBs exert their antihistaminic effects remains unknown. This issue, in addition to the observations that lower concentrations exert opposite effects, necessitates further studies. Nevertheless, this is the first time that JCB compounds have been demonstrated to possess antihistaminic properties.

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