A SIMPLE METHOD FOR MEASURING DEPTH AND DURATION OF ANESTHESIA OF LOCAL ANESTHETICS USING THE MENTAL NERVE BLOCK IN GUINEA PIGS

Shigeo MURAI, Yoshikatsu NAKAMOTO and Tadanobu ITOH

Department of Pharmacology, School of Dentistry, Iwate Medical College, Morioka 020, Japan

Accepted July 31, 1978

The anesthetic potency of local anesthetics has been evaluated by various indexes, such as duration, onset, minimum effective concentration, spread or depth of anesthesia, etc. (1-3). Of these indexes, "depth of anesthesia" is often used as a parameter that expresses the
degree of local anesthesia (4-6). This is an interesting parameter from the clinical point of view, since this is concerned with the problem of whether the best local anesthetic is the one which most deeply abolishes the peripheral sensation or one which abolishes the sensation for the longest period of time (2). In dental practice, the degree of depth of anesthesia produced by various local anesthetics has been estimated from per cent increases in pain threshold ranging from the level of non-anesthesia to complete anesthesia by using the method of Björn (3) in the human dental pulp. In the experimental animal, a similar method with the technique of Björn (3) has been attempted on dogs (4, 5), but there are difficulties when conscious dogs are used (2). Recently, Simard-Savoie (7) reported a simple method for measuring the duration of anesthesia activity of local anesthetics, using the mental and incisive nerve block at the mental foramen in the rat.

We attempted to develop a simple experimental method for measuring depth and duration of anesthesia produced by local anesthetics used in dentistry, using a modification of the method of Björn (3) and Simard-Savoie (7).

Male guinea pigs weighing 300 to 400 g were fixed in a supine position, and bipolar platinum wire electrodes were applied on the oral mucosa proximal to the area of the incisor of the lower mandible. The position of the electrodes applied was approx. 4 to 5 mm apart from the mental foramen. Prior to the injection of test solutions, normal pain threshold for each animal was measured by the application of a variety of strengths of voltage (V) at 10 Hz square pulses with a duration of 0.1 msec for 2 sec, delivered by an electronic stimulator (Nihon Kohden, SEN-3101). The minimum voltage producing at least one of the following pain responses, such as licking, chewing movement or vocalization, was recorded as normal pain threshold. In this study, animals which showed normal pain threshold ranging from 4 to 6 V were used. After the determination of normal pain threshold, a constant volume (0.1 ml) of a test solution was slowly injected at the mental foramen of the lower mandible, using a 26 gauge needle. After the injection, the strength of stimulus was increased until pain responses were observed. If no pain responses were obtained and the tremor of lips or mandible was produced, this stage was regarded as being complete anesthesia and the increase of stimulus was stopped. After the injection, the measurement of pain threshold was made at 2 min intervals for 20 min, and then subsequently at 5 or 10 min intervals until pain threshold fell at the level of normal threshold. Depth of anesthesia was estimated from per cent increases of normal pain threshold. The duration time was calculated by the time from the injection to complete recovery of normal pain threshold. In this study, the commercial solutions of 2% procaine HCl (Daichi), 2% lidocaine HCl (Fujisawa) and 2% mepivacaine HCl (Yoshitomi) were used. The injection of 0.1 ml of a 0.9% saline solution containing epinephrine 1: 50,000 produced no increase in pain threshold. The statistical significance of difference in the results was calculated according to Student’s t-test.

Three local anesthetic solutions, with or without epinephrine 1: 50,000 markedly raised pain threshold within 1 min after the injection. Of the three solutions without epinephrine, the 2% mepivacaine solution raised pain threshold more than the 2% procaine and the 2%
lidocaine solution (p<0.05) and therefore the most potent. There was no difference in increase in pain threshold when the 2% procaine and the 2% lidocaine solution were used. Although the duration of anesthesia of the 2% mepivacaine solution was significantly longer than that of the 2% procaine and the 2% lidocaine solution (p<0.05), the potency of all three solutions without epinephrine diminished within approx. 20 min after injections.

![Graph showing increase in pain threshold with application of different solutions](image)

**Fig. 1.** Increase in pain threshold with application of a 2% procaine, a 2% lidocaine and a 2% mepivacaine solution, with or without epinephrine 1:50,000 on the mental nerve block in guinea pigs. Numbers in parentheses indicate the number of experiments. A 0.1 ml of each solution was administered. Dotted areas indicate change in increase of pain threshold of 2% solutions without epinephrine. epi: epinephrine, Mean±S.E. For explanation of testing procedure see text.

**Table 1.** Local anesthetic activities tested by the mental nerve block in guinea pigs

<table>
<thead>
<tr>
<th>Agents</th>
<th>No. of animals</th>
<th>Normal pain threshold before injection (volt)</th>
<th>Maximal pain threshold after injection (volt)</th>
<th>Increase in pain threshold (%)</th>
<th>Duration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% procaine</td>
<td>10</td>
<td>4.2±0.1</td>
<td>14.8±0.7</td>
<td>356±22</td>
<td>14.0±0.7</td>
</tr>
<tr>
<td>2% lidocaine</td>
<td>8</td>
<td>4.3±0.2</td>
<td>17.5±0.7</td>
<td>415±59</td>
<td>13.5±1.1</td>
</tr>
<tr>
<td>2% mepivacaine</td>
<td>8</td>
<td>4.1±0.1</td>
<td>24.8±1.4</td>
<td>603±36</td>
<td>18.5±1.8</td>
</tr>
<tr>
<td>2% procaine with epinephrine 1:50,000</td>
<td>10</td>
<td>4.5±0.2</td>
<td>28.5±1.1</td>
<td>639±28</td>
<td>122.0±7.6</td>
</tr>
<tr>
<td>2% lidocaine with epinephrine 1:50,000</td>
<td>6</td>
<td>4.5±0.2</td>
<td>33.5±1.2</td>
<td>753±41</td>
<td>125.0±12.3</td>
</tr>
<tr>
<td>2% mepivacaine with epinephrine 1:50,000</td>
<td>6</td>
<td>4.3±0.2</td>
<td>31.3±1.8</td>
<td>728±47</td>
<td>95.0±10.6</td>
</tr>
</tbody>
</table>

1) Levels of normal pain threshold before injection = 100%
2) Time from injection to complete recovery of normal pain threshold before injection. Mean±S.E.
The addition of epinephrine 1:50,000 to the 2% procaine and the 2% lidocaine solution further raised pain threshold and prolonged the duration of anesthesia in comparison with 2% solutions without epinephrine, whereas the addition of epinephrine to the 2% mepivacaine solution increased markedly the duration of anesthesia, but produced no significant increase in pain threshold when compared with the 2%, mepivacaine solution without epinephrine (p<0.05). In these solutions containing epinephrine, there was no significant difference between the value in the duration of anesthesia nor in the increase in pain threshold (p<0.05). These results are shown in Fig. 1 and Table 1.

In the present study of depth and duration of anesthesia of local anesthetic solutions, except for the depth of anesthesia of mepivacaine solution, all markedly increased with addition of epinephrine. Truant et al. (8) stated that in the oral cavity with a high vascularity, local anesthetics required the addition of vasoconstrictors in order to provide adequate depth of anesthesia. Monheim (9) also reported that vasoconstrictors used in dentistry not only prolonged but also increased the intensity of anesthesia. Our present results are in parallel to these findings. In this study, the addition of epinephrine to the mepivacaine solution increased only the duration of anesthesia. This result of mepivacaine is in agreement with findings of Luduena et al. (10), who used intradermal anesthesia in guinea pigs. These workers reported that addition of epinephrine to mepivacaine solution increased considerably the duration of action, but produced only a slight increase in activity. This distinct property of mepivacaine may be partially due to its vasoconstricting effects (11).

Increases in pain threshold produced by the three solutions with epinephrine 1:50,000 showed similar patterns. This result may well explain the complete anesthesia which was achieved using these solutions.

Since the duration of anesthesia is not necessary parallel to depth of anesthesia (5), it may be valuable to measure depth and duration of anesthesia of local anesthetics simultaneously. Since the present results are supported by views of others (8, 9, 10), it is suggested that the present method outlined herein may be useful for evaluating depth and duration of anesthesia of local anesthetics used in clinical dental practice.

REFERENCES
SHORT COMMUNICATIONS  Japan. J. Pharmacol. 28, 925 (1978)


EFFECTS OF N-MERCAPTOACYLAMINO ACIDS ON INHIBITION OF ANGIOTENSIN I CONVERTING ENZYME

Yoshihiko FUNAE, Tadamitsu KOMORI, Daizo SASAKI and Kenjiro YAMAMOTO
Laboratory of Chemistry and *Department of Pharmacology, Osaka City University Medical School, Abeno-ku, Osaka 545, Japan
Accepted August 28, 1978

Angiotensin I converting enzyme (ACE) [peptidylpeptide hydrolase, EC 3.4.15.1] converts angiotensin I (Ang I) to angiotensin II by releasing the C-terminal sequence residue (His-Leu-OH) of the decapetide. This enzyme is a zinc containing metalloenzyme (1). It is well known that the activity of this enzyme is inhibited by chelating agents such as EDTA, 8-hydroxyquinoline (8-OQ) and SH-compounds [2,3-mercapto-1-propanol (BAL), cysteine, 2-mercaptoethanol, dithiothreitol (DTT)] (2–5). Previously we reported that 2-mercapto-propionylglycine (tiopronin) has the ability to form a tight complex with zinc and other metals (6), and that this SH-compound has a hypotensive effect in rats (7).

In the present experiment, we studied the inhibitory effect of several newly synthetized SH-compounds on ACE activity in vitro. SH-compounds derived from tiopronin are (4R)-3-[(2S)-3-mercapto-2-methylpropanoyl]-4-thiazolidinecarboxylic acid (SA 291), (2S)-N-[(2S)-2-mercapto-2-propanoyl] tryptophan (SA 219) and (4R)-3-[(2S)-2-mercapto-2-propanoyl]-4-thiazolidine carboxylic acid (SA 300). ACE activity was determined using two substrates. When we used hippuryl-L-histidyl-L-leucine (HHL) as substrate, the enzyme activity was measured by the method of Cushman and Cheung (3). When Ang I was used as substrate, 0.1 ml of Ang I (1,000 ng/ml), 0.1 ml of ACE (40 mU/ml) and 0.8 ml of 0.1 M phosphate buffer (pH 8.3) containing 0.3 M NaCl were incubated 30 min. at 37°C. Ang I was measured by radioimmunoassay using a commercial assay kit. ACE was extracted from rabbit lung by the method of Dorer et al. (8) and purified further by DEAE-Sepharose chromatography. The specific activity of the present preparation was 0.14 units per mg protein using HHL as substrate. To determine the affinity constant (Km) and mode of inhibition, ACE activity was measured at various concentrations of HHL (0.2–5.0 mM) with or without SH-compounds and a Lineweaver-Burk plot was made.

Concentration of test compounds producing 50% inhibition of ACE (ID50) is shown in Table I. The values of ID50 of conventional inhibitors of ACE such as EDTA, BAL and