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LOCAL ANESTHETIC ACTIVITY OF *P*-AMINO BENZO HYDROXAMIC ACID IN COMPARISON TO BENZOCAINE

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Abstract: **Introduction:** Local anesthetics are agents that block the sensory transmission from a local area of the body to the central nervous system; it is believed that the main mechanism of action of local anesthetics is associated with the blocking of sodium channels. Hydroxamic acids fulfill a variety of roles in biology and medicine. The aim of this work was to prepare *p*-aminobenzohydroxamic acid and to evaluate its expected local anesthetic activity in comparison to benzocaine. **Materials and Methods:** *p*-Aminobenzohydroxamic acid was prepared from *p*-aminobenzoic acid. Ferric chloride test, melting point, nitrogen content and IR spectroscopy achieved identification of *p*-aminobenzohydroxamic acid. The prepared compound was tested for local anesthetic activity in a comparative study with the standard local anesthetic agent benzocaine using the foot withdrawal reflex model. **Results:** *p* Aminobenzohydroxamic acid at doses 40, 200, and 1000 µg showed 33.93 ± 0.80 % , 56.38 ± 0.87 % and 40.31 ± 0.74 % respectively, local anesthetic activity as measured by percent delay withdrawal time. Benzocaine at doses 40, 200, and 1000 µg showed 13.48 ± 0.96 % , 25.74 ± 0.91% and 44.12 ± 1.20 % respectively, local anesthetic activity as measured by percent delay withdrawal time. **Discussion & Conclusion:** Benzocaine, which was used in this study as a standard local anesthetic agent, caused a dose dependant delay in the foot withdrawal reflex of the frog. Such results were not observed with *p*-aminobenzohydroxamic acid. It appears that the local anesthetic effect of the later was not dose dependant. It is worth noting that *p*-aminobenzohydroxamic exhibited relatively potent local anesthetic activity at small doses when compared to benzocaine.

Keywords: *p*-Aminobenzohydroxamic acid, Benzocaine, local anesthetic



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INTRODUCTION

Local anesthetics are agents that block the sensory transmission from a local area of the body to the central nervous system [1].

Various theories have been forwarded to explain their mechanism of action [2]. However, it is believed that the main mechanism of action of local anesthetics is associated with the blocking of sodium channels [3]. It has been shown that benzocaine blocks two different types of sodium channels [4]. Since the drug molecule must cross the lipid membrane to reach the cytoplasm, the more lipid soluble form reaches effective intracellular concentrations more rapidly than the ionized form. On the other hand, once inside the axon, the ionized form of the drug appears to be a more effective blocking entity [1].

Infiltration anaesthesia is the most common form of regional anaesthesia and involves subcutaneous injection of small volumes of local anaesthetic solution into the tissues. By this method, the nerve fibres at the site of operation are desensitized [5].

Hydroxamic acids fulfill a variety of roles in biology and medicine. They have antibacterial and antifungal properties among other biological activities [6]. Furthermore, hydroxamic acids are very weak acids; their pKa values ranging from 7.05 for nitrobenzohydroxamic acid to 11.33 for N-phenylbutrylhydroxamic acid [7]. Accordingly, the hydroxamic acids ionize only in highly alkaline media. To the best of our knowledge, no attempts were made to investigate the effect of the hydroxamic acid moiety on the local anesthetic activity of neither ester-type nor amide-type local anesthetic drugs. It was hoped that conversion of *p*-aminobenzoic acid into the corresponding hydroxamic acid would result in an isostere group to the ester moiety that present in benzocaine [8]. Hence, this might offer a good local anesthetic activity. The aim of this work was to prepare *p*-aminobenzohydroxamic acid and to evaluate its expected local anesthetic activity in comparison to benzocaine.

MATERIALS & METHODS

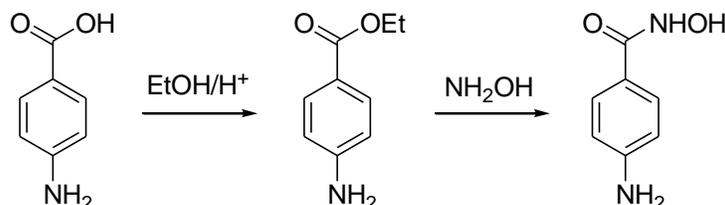
Preparation of ethyl-*p*-aminobenzoate

Absolute ethanol (100 ml) was placed in a round-bottomed flask, and then *p*-aminobenzoic acid (13.7 g, 0.1 mole) was added followed by concentrated sulphuric acid (14 ml). The mixture was refluxed for 2 hours. The hot solution was then poured into excess of cold water contained in a beaker. Sodium carbonate was added to the clear solution until it was neutral to litmus paper. The precipitate was filtered off at the pump, dried in air, and recrystallized from rectified spirit.

Preparation of *p*-aminobenzohydroxamic acid

Sodium hydroxide (25%, 80 ml) was added to a mixture of hydroxylammonium sulphate (13.2 g, 0.1 moles) and ice (100 g) contained in 500ml conical flask. To this was added sodium sulphate

(2 g) followed by the ester (16.5 g., 0.1 moles). The flask was then covered and the mixture stirred at room temperature for 48 hours. The mixture was acidified to pH 6 using sulphuric acid (25%), allowed to cool and the aqueous solvent was evaporated under reduced pressure. The residue was extracted with hot methanol and filtered. Methanol was evaporated and the crude hydroxamic acid was dissolved in hot water, treated with charcoal, and recrystallized (m.p.185^o, Lit 180-185^oC).



Scheme 1: Synthesis of p-aminobenzohydroxamic acid

Foot withdrawal reflex of the frog

A frog was killed by stunning and decapitating the head. The viscera were removed from the abdominal pouch with fine forceps with care not to damage the nerves at the base of the spinal cord. The frog was spinned by its front limbs to a vertically mounted corkboard so that the hind legs were suspended freely below it. Both hind limbs were dipped into a beaker containing 0.05 M HCl, and the time taken for the frog to withdraw the feet was noted. Legs were washed with 0.9% normal saline after feet withdrawal reflex [9].

RESULTS

p-Aminobenzohydroxamic acid was prepared (Scheme 1) according to the method outlined by Van't [10]. FeCl₃ test [11], m.p, nitrogen content, and IR spectrum [12] confirmed the structure of the prepared compound.

p-Aminobenzohydroxamic acid at doses 40, 200, and 1000 µg showed 33.93 ± 0.80 % , 56.38 ± 0.87 % and 40.31± 0.74 % respectively, local anesthetic activity as measured by percent delay withdrawal time (table 1) .

Benzocaine at doses 40, 200, and 1000 µg showed 13.48 ± 0.96 % , 25.74 ± 0.91% and 44.12 ± 1.20 % respectively, local anesthetic activity as measured by percent delay withdrawal time (table 1).

Table 1: Time required for feet withdrawal reflex after treatment with p-aminobenzohydroxamic acid (PABHA) and benzocaine (n = 4).

Group	Drug	Treatment (µg)	Withdrawal time (sec) mean ± SEM	Percent delay
1	PABHA	1000	5.50 ± 1.07	40.31 ± 0.74
2	PABHA	200	6.13 ± 1.20	56.38 ± 0.87
3	PABHA	40	5.25 ± 1.13	33.93 ± 0.80
4	Benzocaine	1000	5.88 ± 1.46	44.12 ± 1.20
5	Benzocaine	200	5.13 ± 1.17	25.74 ± 0.91
6	Benzocaine	40	4.63 ± 1.22	13.48 ± 0.96

Control times for feet withdrawal as mean ± SEM were:

3.92 ± 0.67 seconds for PABHA & 4.08 ± 0.74 seconds for Benzocaine.

DISCUSSION

Local anesthetics are agents that block the sensory transmission from a local area of the body to the central nervous system [1]. Without the use of local anaesthetics effective and reversible regional block will not be possible [13].

p-Aminobenzohydroxamic acid (1000 µg) displayed 40.31± 0.74 % local anesthetic activity as measured by percent delay withdrawal time [9]. At the same dose, benzocaine showed an activity of 44.12 ± 1.20 %. From these results, it can be concluded that at dose as high as 1000 µg, *p*-aminobenzohydroxamic acid showed similar potency to benzocaine. It was noted that at a dose of 200 µg, the activity of benzocaine almost was 50 % lower (25.47 ± 0.91%) than that at the high dose of 1000 µg of the drug. On the other hand, PABHA showed a reversed effect where at the dose of 200 µg it resulted in a higher response (56.38 ± 0.87 %), than at the higher dose of the drug. Smaller dose (40 µg) of benzocaine caused a dramatic reduction in its activity giving a response of 13.48 ± 0.96 %. The corresponding activity of *p*-aminobenzohydroxamic acid with a dose of 40 µg was 33.93 ± 0.80 % (Table 1). From these results, it is evident that benzocaine, which was used in this study as a standard local anesthetic agent, caused a dose dependant delay in the foot withdrawal reflex of the frog. Such results were not observed with *p*-aminobenzohydroxamic acid. It appears that the local anesthetic effect of the later was not dose dependant. It is worth noting that *p*-aminobenzohydroxamic exhibited relatively potent local anesthetic activity at small doses when compared to benzocaine. This observation could not be explained. It could be postulated that the dose required to cause optimum effect is critical. However, further evidence is required to confirm this postulation.

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