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Antagonistic Effect of Benzalkonium Chloride on Nicotine Induced Contractions

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ABSTRACT

The persent study aimed to determine potential of Benzalkonium chloride (BKC) antagonistic effect against nicotine induced contractions using isolated frog's rectus abdominis muscle. This gives us information about the potential use of BKC in treatment of spastic disorders and in nicotine toxicity. BKC, also known as alkyldimethylbenzylammonium chloride and ADBAC, is a cationic surface-acting agent belonging to the quaternary ammonium group. The very important features of benzalkonium salts are their bactericidal and antimicrobial properties. For these reasons they are widely used as preservatives for ophthalmic, nasal and parenteral products. Graded dose response of the nicotine (contractions) on frog's rectus abdominis muscle was recorded to identify the submaximal dose. Then Submaximal dose (160µg) along with graded dose of BKC (1,2,4,8,16,32,64,128µg) was given to determine the effect of BKC on contractions induced by nicotine. PA_2 value of the BKC against nicotine was determined. From the results it found that BKC produces dose dependent antagonistic effect (sketeltal muscle relaxing effect) on contractions produced by the nicotine. 120µg of BKC blocks the effect of 160µg of nicotine and PA_2 value was found to be 1.5µg. Finally, it was concluded that BKC has potential to block the effect of nicotine i.e. it has potential skeletal muscle relaxant effect.

Keywords: Antagonistic effect, Benzalkonium chloride, and Nicotine

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INTRODUCTION

Skeletal muscle is a form of striated muscle tissue which is under the control of somatic nervous system; that is to say, it is voluntarily controlled. These muscles are made up of functional and structural unit sarcomere which is made up with actin and myosin structural and troponin and tropomyosin regulatory proteins for the muscle contraction to occur. Skeletal muscle cells are excitable and are subject to depolarization by the neurotransmitter acetylcholine (ACh), released at the neuromuscular junction by motor neurons [1]. ACh acts as an excitatory neurotransmitter at neuromuscular junctions in skeletal muscle [2]. The two main classes of ACh receptors are nicotinic receptors (nAChRs) which is stimulated by nicotine (agonist) and muscarinic receptors (mAChRs) which is stimulated by muscarine (agonist).

Nicotinic AChRs are ionotropic receptors, permeable to sodium, potassium, and calcium ions. They are stimulated by nicotine and ACh which are not structurally similar compounds. Recent research studies provide an information that ACh acts on nicotinic muscular receptors (N_MRs) and nicotine acts on ryanodine receptors (RyRs) [3]. They are of two main types, muscle-type and neuronal-type. The former can be selectively blocked by curare and the later by hexamethonium. The main location of nAChRs is on motor end plates of muscle, on autonomic ganglia (both sympathetic and parasympathetic), and in the CNS [4]. Hyperactivity of the motor neurons or excess release of ACh leads to spastic disorders [5].

Benzalkonium chloride (BKC), also known as alkyldimethylbenzylammonium chloride and ADBAC, is a cationic surface-acting agent belonging to the quaternary ammonium group [6]. The chemical is a nitrogenous mixture of alkylbenzyldimethylammonium chlorides of various even-numbered alkyl chain lengths [7]. The very important features of benzalkonium salts are their bactericidal and antimicrobial properties. For these reasons they are widely used as preservatives for ophthalmic, nasal and parenteral products. They are also used as topical antiseptics and disinfectants for medical equipment. It has three main categories of use: as a biocide, a cationic surfactant and phase transfer agent in the chemical industry. Topical use of BKC causes corneal neurotoxicity, inflammation and reduces aqueous tears production [8]. It also causes inflammation of nasal mucosa while using as nasal drops for the treatment of allergic rhinitis and rhinitis medicamentosa [9]. The persent study aimed to determine potential of BKC antagonistic effect against nicotine induced contractions using isolated frog's rectus abdominis muscle. This gives us information about the potential use of BKC in treatment of spastic disorders and in nicotine toxicity.

MATERIALS AND METHODS

Chemicals

Nicotine used in the study was obtained from Central Tobacco Research Institute, Rajahmundry as a gift sample. BKC procured from Sidhi Chemicals, Visakhapatanam. Sodium dihydrogen phosphate (NaH_2PO_4) was procured from Sd Fine-chem Ltd, Mumbai. Sodium

chloride (Nacl), Potassium Chloride (Kcl), Calcium Chloride ($CaCl_2$), Sodium bicarbonate ($NaHCo_3$) and Glucose were procured from Qualigens Fine Chemicals, Mumbai, India. And all other chemicals used in the study were of analytical grade.

Animals Frog (Rana tigrina)

Physiological Solution Frog's Ringer Solution

Equipments & Instruments Sherrington recording drum, Students organ bath, Simple Lever, Lever holder and Aerator

Preparation of Frog's Ringer solution

NaCl (6.5g), KCl(0.14g), $CaCl_2$(0.12g) and glucose(2g) were accurately weighed and dissolved in distilled water and was made to 900 ml. $NaHCO_3$(0.2g) dissolved in distilled water was added slowly to the above solution by mixing and made to 1 L by distilled water. The final pH of the solution was adjusted and maintained at 7.4 by the addition of sodium hydroxide.

Preparation of drug solutions:

Benzalkonium chloride solution: 1ml of 50% solution of BKC is added to 100ml of frog Ringer solution and from that 1ml is made to 100ml with frog Ringer solution. Again from that solution, 1ml is taken and made to 50ml. This gives a concentration of 1000µg solution.

Nicotine solution: 100µl (1, 01, 000 µg; density – 1.01 g/cm^3) of pure nicotine was taken and was dissolved in distilled water and made to 101ml.

Isolation & mounting of rectus abdominis muscle of frog

A frog (Rana tigrina) was pithed by inserting pithing needle into the occipito atlantic junction or foramen magnum and destroying the brain and spinal cord. The skin on the abdomen was removed and the rectus abdominis muscle was exposed. It was separated from other muscle attachments. A midline incision on the abdominal muscle was made from pelvic girdle to pectoral girdle and two muscle preparations were identified (Ghosh, 2005). Two threads were sewn to the top and bottom of each muscle preparation before detaching it from the body of the frog. The rectus abdominis muscle from the body of frog was detached and mounted in up-right position in the organ bath containing frog Ringer solution with a tension of 1g.The organ bath was aerated and the tissue was stabilized for 45 min, during which period the tissue was washed with fresh quantum of Ringer for atleast four times [10,11].

Study design

The graded dose responses due to nicotine were recorded on the frog's muscle. The muscle was left for relaxation until the lever comes to the baseline position during which the

muscle was washed with fresh quantum of ringer each time. Responses to increasing doses of nicotine were recorded until ceiling response was observed. A response on the sensitive region of the DRC was selected such that it is about 75% of the ceiling response and it is taken as submaximal response. The effect of initial dose of BKC was seen on submaximal response of nicotine. The muscle was treated with BKC for 30sec and then the submaximal response of nicotine was recorded. Similarly the doses of BKC were increased gradually namely 1 µg, 2 µg, 4 µg, etc. to find their influence on the response of submaximal dose of nicotine.

The submaximal response of nicotine was repeated in the end to check the consistency in its response when tried alone. The graph was fixed with a fixing solution. The heights of the responses were measured and the results are given in the table no 1 and 2

Determination of PA_2 value

PA_2 value is defined as the negative logarithm of the molar concentration of antagonist, in the presence of which double the dose of agonist is required to produce the same effect as produced in absence of antagonist.

$$PA_2 = - \text{antilog } M$$

PA_2 value is useful in differentiating the nature of receptors involved in particular drug action. PA_2 value for antagonist is always same, irrespective of the agonist used. Higher the PA_2 value, more potent is the antagonist. An antagonist acting on the same receptor will have same PA_2 value in all the tissue or organ preparations.

Procedure to determine PA_2 Value

The dissection and mounting of rectus abdominis muscle was made as described previously. Responses to increasing doses of nicotine were recorded until ceiling response was observed. Two doses bearing 1:2 dose ratio and eliciting submaximal responses (A, 2A) were selected for PA_2 determination say 20 µg and 40 µg respectively. The tissue was standardized with the selected doses of Nicotine. A tissue was said to be standardized when it responds identically to the same dose of an agonist when repeated. The concentration due to the double dose of Nic (40 µg) was recorded in the presence of varying concentrations (1 µg, 2 µg, 4 µg etc.) of BKC. The response due to double the dose of Nic (40 µg) i.e., before adding BKC, was considered as 100% response. The corresponding percent response to the double dose of Nic (40 µg) in the presence of varying concentrations of BKC were determined. A graph was plotted representing negative logarithm of molar concentration of BKC employed along X-axis and % response along Y-axis. The PA_2 value for BKC was read out from the graph directly (Fig.1). It corresponds to the % response obtained with half the dose of Nicotine [12].

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RESULTS AND DISCUSSION

Antagonistic effect

Nicotine produced dose dependent response on isolated rectus abdominis muscle of frog. The amplitude of the initial contractile response against a dose of 10 µg was 1mm. The ceiling response of nicotine was found to be 70 mm at 640 µg. The submaximal dose of nicotine selected was 160 µg. BKC was found to block the submaximal response of nicotine on frog skeletal muscle. The dose required to produce such effect was 128 µg. Results shown on Table 1 and Percentage change of nicotine response in the presence of BKC on normal rectus abdominis muscle of frog (N=5) was showed in Table 2.

PA_2 Value determination

Nicotine produced dose dependent response on isolated rectus abdominis muscle of frog. The amplitude of the initial contractile response against a dose of 10 µg was 12mm. The ceiling response of nicotine was found to be 70 mm at 80 µg. The two doses selected were 20 µg and 40 µg and their responses obtained were 24 mm and 40 mm respectively. BKC was found to decrease the response of double dose of nicotine (40 µg) to that of single dose (20 µg) on frog skeletal muscle. The dose required to produce such effect was 4 µg. From the graph plotted with the values of table 3. The -log M value was found to be 0.185 (figure 1) and from the calculation PA_2 calculated as 1.53 µg.

Table 1. Antagonistic effect of benzalkonium chloride on nicotine(Nic) induced contractions on isolated rectus abdominis muscle of frog (N=5)

Dose of BKC + Nic160 µg (µg)	Height of the response in different trials (mm)						Mean ± SD
	T_1	T_2	T_3	T_4	T_5	Mean	
Control (Nicotine 160 µg)	50	48	45	53	55	50.20	50.20 ± 3.96
1	43	40	35	42	45	41.00	41.00 ± 3.80
2	37	36	34	40	40	37.40	37.40 ± 2.60
4	36	34	32	37	38	35.40	35.40 ± 2.40
8	35	32	30	36	37	34.00	34.00 ± 2.91
16	25	24	18	32	30	25.80	25.80 ± 5.49
32	12	10	9	16	18	13.00	13.00 ± 3.87
64	6	7	4	8	9	6.80	6.80 ± 1.92
128	3	4	1	4	6	3.60	3.60 ± 1.81

CONCLUSION

BKC a preservative used in eye and nasal drops blocked the action of nicotine. Hence it can be used as skeletal muscle relaxant, spastic disorders. It can also be used as a potential antagonist in nicotine toxicity.

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Table 2. Percentage change of nicotine response in the presence of benzalkonium chloride on normal rectus abdominis muscle of frog (N=5)

Dose of BKC + Nic160 µg (µg)	Percentage reduction of response in different trials (%)						Mean ± SD
	T_1	T_2	T_3	T_4	T_5	Mean	
1	-14.00	-16.66	-22.22	-20.75	-18.18	-18.32	-18.32 ± 3.26
2	-26.00	-25.00	-24.44	-24.52	-27.27	-25.49	-25.49 ± 1.19
4	-28.00	-29.16	-28.88	-30.18	-30.90	-29.48	-29.48 ± 1.12
8	-30.00	-33.33	-33.33	-32.07	-32.72	-32.27	-32.27 ± 1.36
16	-50.00	-50.00	-60.00	-39.62	-45.45	-48.60	-48.60 ± 7.49
32	-76.00	-79.16	-80.00	-69.81	-67.27	-74.10	-74.10 ± 5.68
64	-88.00	-85.41	-91.11	-84.90	-83.63	-86.45	-86.45 ± 2.97
128	-94.00	-91.66	-97.77	-92.45	-89.09	-92.82	-92.82 ± 3.21

Table 3. Logarithmic values of benzalkonium chloride and corresponding % response

Molar concentration of BKC (M)	- log M	Response, R (mm)	% R
1 µg + 40 µg Nic	0	24	60.00%
2 µg + 40 µg Nic	- 0.3010	17	42.50%
4 µg + 40 µg Nic	- 0.6020	11	27.50%

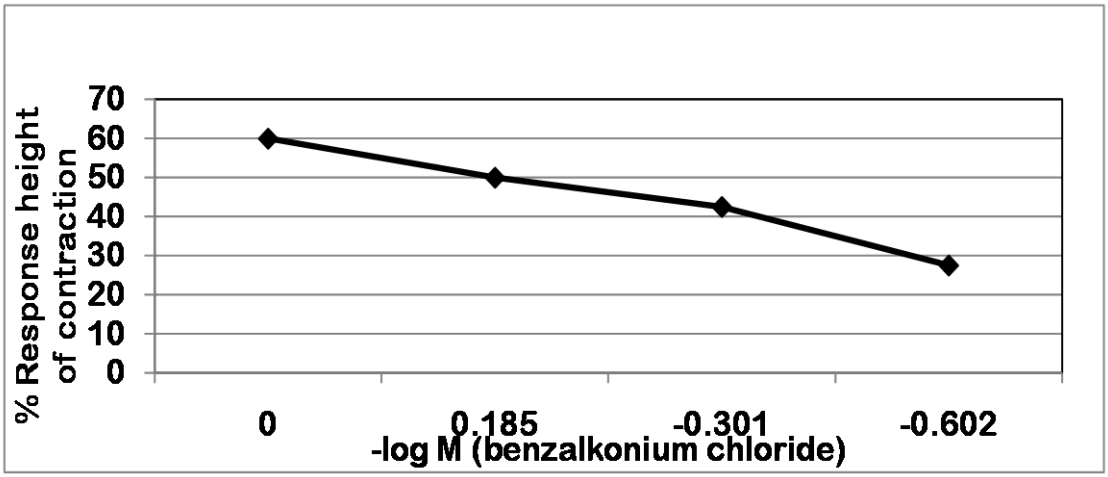

Figure1. Determination of PA_2 value of benzalkonium chloride on nicotine on rectus abdominis muscle of frog

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