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ASSESSMENT OF LOCAL ANESTHETIC ACTIVITY OF LIGNOCAINE BY SIMULTANEOUS ADMINISTRATION OF POTASSIUM CHANNEL AGONISTS NICORANDIL IN ALBINO RATS

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ABSTRACT: There are reports about a possible weak local anaesthetic activity of nicorandil, a potassium channel agonist (PCA). In addition**,** modification of local anesthetic activity of lignocaine by PCA is not clearly defined. The objective of the present study is to evaluate local anesthetic activity of nicorandil and to evaluate the influence of nicorandil on the efficacy and duration of local anesthesia by lignocaine. A total number of 84 albino rats of either sex were divided into 14 groups of 6 animals each. Two methods, sciatic nerve blockade and tail clip method were applied for the study. Control group received 0.2ml normal saline, whereas the study group received 0.2ml of 1% drug solution (lignocaine or nicorandil) combined with 0.9% sodium chloride.

The results showed weak local anaesthetic activity with the higher dosages of nicorandil (10 mg/kg, 100mg/kg). Combination of nicorandil (1mg/kg, 10mg/kg and 100mg/kg) and lignocaine (5mg/kg) yielded synergistic results like decreased onset latency and prolonged reaction time. To conclude unquestionably there are multiple mechanisms involved for causation of local anesthetic effect by drugs like lignocaine. PCAs like nicorandil will influence the activity of these drugs as observed in the present study. Mechanism of this may be complex. Nicorandil may increase the plasma membrane permeability to potassium causing hyper polarization and moves the membrane potential away from the threshold required to generate an action potential.

Key words: lignocaine, nicorandil, local anesthetic, potassium channel agonist.

INTRODUCTION

Local anesthesia is useful in a wide variety of clinical situations. It is warranted whenever a clinical procedure causes pain that could be eliminated by the use of local anesthetics. It increases patient's comfort and facilitates patient's cooperation during procedure.

Local anesthesia in peripheral nerve is produced by blockade of voltage gated sodium currents is already established (Elliot JR, 1989, Hille, 1992). The effect of local anesthetics (LAs) upon delayed rectifier potassium (K^+) current is of minor importance (Srichartz, 1987, Arhem, 1974). Further, the contributions of potentially activated K+ currents to the action potential in mammalian fibers are almost negligible (Vogel, 1995) Blockade of the potassium channel might need depolarization to an increased amount of inactivated sodium (Na^+) channels thus blocking impulse propagation (Raymond, 1987)

After the description of first local anesthetic cocaine by Niemann in 1860 from the leaves of Erythroxylon coca bush, a number of clinically useful drugs have been added to the list. Out of these, lignocaine, synthesized in 1943, is still the most widely used local anesthetic.

Potassium channel agonists (PCAs) are smooth muscle relaxants which act by opening potassium channels not only in smooth muscles but also in other excitable tissues causing hyperpolarisation of cell membrane leading to reduction in the intracellular calcium

They have been used in hypertension; angina pectoris etc. and have potential use in other conditions caused by smooth muscle contraction like bronchial asthma and urinary incontinence (Martindale, 2005)

In certain clinical situations, use of local anesthetics in the patients who are on PCA medication is warranted. There are studies exploring possible modification of local anesthetic activity of bupivacaine by PCAs (Gantenbein, 1996). But the modifications of local anesthetic activity of lignocaine by PCAs are not clearly defined. In addition to this there are scanty reports of nicorandil, a PCA, having weak local anesthetic activity (Lacoussaye, 1993, Attolini L, 1996)

This prompted us to undertake the present study of local anesthetic activity of nicorandil and modification of local anesthetic activity of lignocaine by concurrent administration of nicorandil. The outcome may further widen the horizon of understanding the activities of above said drugs which may help towards more rationalization of their clinical use.

MATERIALS AND METHODS

Experimental animals

Wistar albino rats of either sex weighing 150-200 gm were selected and obtained from the central animal house of J.J.M Medical College, Davangere. The rats selected were previously unused for any other experiment. The animals were housed under standard conditions with free access to water and food.

Drugs:

Lignocaine pure powder form manufactured by Astrazeneca (No. BK-8-LG-001), India and Nicorandil pure powder form manufactured by Sun pharmaceuticals (No. 303RI82344) were procured from the respective pharmaceuticals. Normal saline was prepared in the pharmacy laboratory, J.J.M Medical College, Davangere. All drugs to be administered to the rats were dissolved in normal saline.

Inclusion criteria:

- Rats weighing150-200gm of either sex.
- Animals that walk normally with four limbs on the top and inverted side of a wire mesh.
- Animals with a reaction time less than 6 seconds in tail clip.

Exclusion criteria:

- Animals weighing more than 200gms and less than 150gms
- Pregnant and recently delivered animals
- Animals which cannot walk properly with four limbs.
- Animals with a reaction time more than 6 seconds in tail clip.

METHODS

A total number of 84 albino rats (n=84) were divided into 14 group of six animals each. The drug solutions were used in the following dosage.

1. Normal saline as control.
2. Lignocaine 5mg/kg as therapeutic dose
3. Nicorandil 10mg/kg and 100mg/kg.
4. Combination of both the drugs.

All the above injections were given by a 24 to 25 gauge needle attached to a 1ml tuberculin syringe. All drugs used were of 1% concentration in 0.9% sodium chloride solution.

Two methods were used for evaluation

1. Loss of motor control following sciatic nerve blockade of the drug injected limb.
2. Tail clip procedure in which pinching with an artery forceps was done.

Sciatic nerve blockade method:

A total number 7 groups (n=42, 6 in each group) received 0.2mL of the solutions as following:

Group I received – 0.2mL of normal saline (control)
Group II received – lignociane 5mg/kg
Group III received – nicorandil 10mg/kg
Group IV received – nicorandil 100mg/kg

Group V received – lignocaine 5mg/kg + nicorandil 1mg/kg
Group VI received – lignocaine 5mg/kg + nicorandil 10mg/kg
Group VII received – lignocaine 5mg/kg + nicorandil 100mg/kg

All the drugs were injected into the popliteal fossa of right hind limb. The parameters Observed were:

1. Onset latency – the duration between injection of the drug and onset of local anesthetic activity.
2. Ability of the animal to walk. Here the time of recovery from the local anesthetic effect was recorded.

Tail clip method:

A total number 7 groups (n=42, 6 in each group) received 0.2mL of the above said drugs injected subcutaneously bilaterally at the root of the tail .The test for local anesthesia was done by pinching with a polyethylene artery forceps (Attolini L, 1996, Bruguerolle B, 1995).

The parameters observed were:

1. Onset latency:
2. Reaction time – ability of the animal to remove the forceps by biting. Total period of recovery was noted.

As reported in the previous studies (13, 14), the local anesthetic activity in both the methods was evaluated every minute until the onset of activity there after every 5 minutes until the time of recovery. The time of recovery is used as indicative of duration of total anesthetic effect.

Statistical analysis:

One way ANOVA was used for multiple comparisons followed by post hoc Tukey's test for pair wise comparison.

RESULTS

Sciatic nerve blockade method:

The control group (normal saline) had no any alteration in motor activity of the injected limb. Groups that received Lignocaine 5mg/kg (therapeutic dose) showed average onset latency of 8.59 minutes and recovery period of 16.50 minutes. (Table-1)

Table 1 : Comparison of onset latency (onset of action) and recovery period (duration of action) between Gr. II, III and IV by sciatic nerve blockade method

Drug	Gr	Onset latency (in minutes)			Recovery period (in minutes)		
		Mean ±SD	Groups compared	P value	Mean ±SD	Groups compared	P value
Lignocaine 5mg/kg	II	8.592 ± 0.7046	II -III	< 0.0001	16.50 ± 1.871	II -III	0.0320
****Nicorandil 10mg/kg**	III	17.33 ± 0.8987	II-IV	< 0.0001	13.67 ± 2.066	II-IV	1.0000
Nicorandil 100mg/kg	IV	14.07 ± 1.055	III-IV	0.0002	16.50 ± 1.049	III-IV	0.0134

** Local anaesthetic effect of nicorandil in 10mg/kg dose, onset latency was 17.33 minutes more than recovery period 13.67 minutes. This appears to have no significance.

In the present study nicorandil alone has showed weak local anesthetic activity. But the combination of therapeutic dose of lignocaine with various dosages of nicorandil (1mg/kg, 10mg/kg and 100mg/kg) showed different results. Both parameters in lignocaine alone and its combination with nicorandil 1mg/kg were almost comparable. But combination with higher doses of nicorandil yielded low onset latency and prolonged recovery period (3.59 minutes and 1.75 minutes onset latency and 33.0 minutes and 47.0 minutes recovery period respectively). The results are statistically highly significant.

Tail clip method:

The results in this method were almost of same nature as observed with sciatic nerve blockade method. Average onset latency was 9.81 minutes and recovery period 18.83 minutes with therapeutic dose of lignocaine. (Table-2).

Table 2: Comparison of onset latency (onset of action) and recovery period (duration of action) between Gr. II, III and IV by TAIL CLIP METHOD

Drug	Gr	Onset latency (in minutes)			Recovery period (in minutes)		
		Mean ±SD	Groups compared	P value	Mean ±SD	Groups compared	P value
Lignocaine 5mg/kg	II	9.808 ± 0.4152	II –III	< 0.0001	18.83 ± 1.472	II -III	0.1781
Nicorandil 10mg/kg	III	18.43 ± 0.8987	II-IV	< 0.0001	20.33 ± 2.066	II-IV	0.0245
Nicorandil 100mg/kg	IV	14.92 ± 0.9277	III-IV	< 0.0001	22.17 ± 2.714	III-IV	0.2173

* Unpaired t test, p< 0.001 (Highly significant

Nicorandil showed weak anaesthetic activity in this model also. Average onset latency was 14.92 minutes and recovery period 22.17 minutes with nicorandil 100mg/kg body weight.

Combination of higher doses of nicorandil with lignocaine yielded low onset latency and prolonged recovery period (4.64 minutes and 2.88 minutes onset latency and 47.17 minutes and 62.17 minutes recovery period respectively). The results are statistically highly significant. (Table 3 and 4).

Table 3: Comparison of onset latency (onset of action) and recovery period (duration of action) between Gr. V, VI and VII by sciatic nerve blockade method

Drug	Groups	Onset latency (in minutes)			Recovery period (in minutes)		
		Mean ±SD	Groups compared	*P value	Mean ±SD	Groups compared	*P value
Lignocaine (5mg/kg) + Nicorandil (1mg/kg)	V	8.63 ± 0.85	V-VI	< 0.001	15.83 ± 0.46	V-VI	< 0.001
Lignocaine (5mg/kg) + Nicorandil (10mg/kg)	VI	3.59 ± 0.39	V-VII	< 0.001	33.00 ± 0.48	V-VII	< 0.001
Lignocaine (5mg/kg) + Nicorandil (100mg/kg)	VII	1.74 ± 0.51	VI-VII	< 0.001	47.00 ± 0.58	VI-VII	< 0.001
ANOVA		F=189.4 p<0.001, HS			F=119.9 p<0.001, HS		

Figures expressed as Mean ± SD

One way ANOVA, p< 0.001 (Highly significant) **(Post hocTukey's test)**

Table 4: Comparison of onset latency (onset of action) and recovery period (duration of action) between Gr. V, VI and VII by tail clip method

Drug	Groups	Onset latency (in minutes)			Recovery period (in minutes)		
		Mean ±SD	Groups compared	*P value	Mean ±SD	Groups compared	*P value
Lignocaine (5mg/kg) + Nicorandil (1mg/kg)	V	9.56 ± 0.75	V-VI	< 0.001	19.50 ± 2.43	V-VI	< 0.001
Lignocaine (5mg/kg) + Nicorandil (10mg/kg)	VI	4.64 ± 0.34	V-VII	< 0.001	47.17 ± 3.87	V-VII	< 0.001
Lignocaine (5mg/kg) + Nicorandil (100mg/kg)	VII	2.88 ± 0.48	VI-VII	< 0.001	62.17 ± 5.85	VI-VII	< 0.001
ANOVA		F=236.0 p<0.001, HS			F=153.2 p<0.001, HS		

One way ANOVA, p< 0.001 (Highly significant) **(Post hocTukey's test)**

DISCUSSION

Local anesthetics are drugs that produce reversible conduction blockade of impulses along central and peripheral nerve pathways after regional anesthesia. With progressive increase in concentrations of local anesthetics, the transmission of autonomic, somatic sensory and somatic motor impulses is interrupted producing autonomic nervous system blockade, sensory anesthesia and skeletal muscle paralysis in the area innervated by the affected nerve. Removal of local anesthesia is followed by spontaneous and complete return of nerve conduction with no evidence of structural damage to the nerve fibers as a result of drug effects.

Numerous studies performed during the past half a decade have shown that there are complex mechanisms involved in the block of conduction of action potential in excitable cells elicited by local anesthetics:

1. Inhibition of activation of sodium channels as the main mechanism.

2. Majority of local anesthetics have the property to block voltage independent potassium channels in peripheral nerve membranes (Stoelting RK, 2006)

3. It is also postulated that local anesthetics block L-type calcium currents (Collin D, 1999)

The contribution of fast potassium channel block to conduction block of the fiber is small because these channels are mainly responsible for the fast repolarization of action potential. In addition lignocaine reduces the permeability of resting axon to both sodium and potassium ions.

The PCAs are powerful smooth muscle relaxants acting by opening potassium channels primarily in smooth muscle and also in other excitable tissues. Thus these agents are having a capacity to influence activity of local anesthetics. Nicorandil, a pyridine compound has two independent mechanisms (Kazurhi, 1993)

1. It has a niacinamide moiety which opens ATP–dependant potassium channels resulting in potassium ion efflux and hyper polarization of cell membrane.

2. It has a nitrate moiety which activates guanylate cyclase increasing intracellular cyclic GMP concentration. This in turn decreases stored calcium and interferes in calcium activated smooth muscle contraction.

There are few reports of nicorandil possessing weak local anesthetic properties (Gantenbein, 1996, Bruguerolle, 1995) it was observed that the PCAs injected in the region of the sciatic nerve did not induce any alteration of the motor activity of the injected limb (Gantenbein, 1996) and concluded that, decrease in motor activity is due to drug interference and not due to a direct action by these agents.

But in the present study nicorandil has shown decrease in the onset latency and increase in the anesthetic activity in higher dosages in both the animal models (Sciatic nerve blockade and tail clip method). This will suggest that there is some complex mechanism involved at least in the higher dosages of 10mg/kg and 100mg/kg. Combination of therapeutic dose of lignocaine (5mg/kg) with various concentrations of nicorandil yielded different results in both the models. Increase in the dose of nicorandil with lignocaine, reduced onset latency but prolonged the recovery period. Increasing the dose of nicorandil might open more and more potassium channels which may be synergistically acting with the local anesthetic.

Both the PCAs and LAs have pharmacodynamic actions on ion channels which may explain the interference of mechanisms of each other. The observed shortened onset latency and prolongation of anesthetic effects in the present study could be explained by interactions in a direct or indirect way on the same ion channels. Similar conclusions were expressed by the authors of a previous study done with bupivacaine and PCAs (Gantenvein, 1996)

Nicorandil may increase the plasma membrane permeability to potassium causing hyper-polarization and moves the membrane potential away from the threshold required to generate an action potential. The contribution of opening of ATP dependant as well as independent potassium channels may be responsible for this. In addition it has also been postulated that PCAs may influence on the blood flow due to their cardiovascular actions which may lead to variations in local anesthetic uptake.

Since lignocaine is commonly used as a local anesthetic and as a cardiac depressant in arrhythmias, its interaction with co-administration of nicorandil was undertaken.

Lignocaine has effects on central nervous system to produce restlessness, tremors and frank convulsions in addition to cardio-toxicity. There are also reports of PCAs having protective role against the occurrence cardio-toxicity by local anesthetics (Porter JM, 2003). The results of the present study definitely indicate that there is synergistic effect of nicorandil with lignocaine.

To conclude, nicorandil has shown weak local anaesthetic activity and acted synergistically with lignocaine. In order to specify the mechanisms involved, more extensive studies are required. Further, as nicorandil is under investigation for possible use in various cardiovascular conditions, cerebral ischemia, heart failure, and erectile dysfunction. Apart from use in hypertension and angina pectoris, the finding of the studies are helpful in more rationalizing the use of both lignocaine and nicorandil.

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