ORIGINAL RESEARCH PAPER

Pharmacology

NICORANDIL, AN ADENOSINE TRIPHOSPHATE SENSITIVE POTASSIUM CHANNEL OPENER, INDUCED BY INTRAVENOUS PROPOFOL ANESTHESIA, MOTOR CO-ORDINATION PROPERTIES IN RODENT MODELS

KEY WORDS: Adenosine triphosphate sensitive potassium channels openers, nicorandil, propofol, endothelin -1, Glibenclamide, grip strength test.

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ABSTRACT

OBJECTIVE: The objective of this study was the effects of an adenosine triphosphate sensitive Potassium channel opener nicorandil may suppress or induced anesthetic action induction of propofol, on motor performance in mice.

MATERIAL AND METHODS: 48 Swiss Albino mice were included in the study in 8 groups of 6 animals in each (n=6). Treatment groups are solvent control, Methohexital alone, Propofol alone, Pretreated nicorandil with Propofol, nicorandil with PLC inhibitor Endothelin-1 , pretreated Endothelin-1 with Propofol, Pretreated with nicorandil, Endothelin-1 with Propofol , Pretreated with potassium channel blocker glibenclamide with Propofol.

RESULTS: The effect of Adenosine triphosphate sensitive Potassium channel opener nicorandil with propofol on muscle function the present study demonstrates that significantly Enhanced muscle function, as evidenced by increased grip strength, as well as Decreased fatigue. Potassium channel opener nicorandil Partialy inhibition the muscle strength the grip strength compared with propofol alone. P<0.01** (Forelimb, 86.6± 6.2, Hindlimb 74.8± 4.0) Grip strength of the mice Propofol alone treated was significantly inhibitied. Grip strength test showed increase of (a) forelimb and (b) hindlimb grip strength in the mice treated with the nicorandil with propofol. A decline in muscle strength has been reported potassium channel blocker with propofol.

CONCLUSIONS: The effects of propofol on the endothelin-1-induced activation of muscle function. KATP channels regulate the membrane potential, which controls calcium entry through voltage-dependent calcium channels, and thereby contractility through changes in intracellular calcium. Present study have suggested that opening KATP channels in vascular smooth muscle causes vasodilatations

INTRODUCTION

The anti-anginal agent nicorandil is known as an opener of the ATP-sensitive potassium (K^+ATP) channel with a nitrate moiety[1]. A significant amount of clinical evidence has demonstrated that nicorandil protects the heart against ischemic injury, improves the recovery of post-ischemic contractile dysfunction and can reduce infarct size in several animal models [2]Nicorandil is an adenosine triphosphate sensitive potassium (K^+ ATP) channel opener. The K^+ ATP channel openers induce cell hyperpolarization, thus, resulting in a decrease in the intracellular calcium ion (Ca^{2+}) level and neurotransmitter release which account for antinociception[3,4,5].

Propofol (2, 6-diisopropylphenol) is a potent intravenous hypnotic agent which is widely used for the induction and maintenance of anesthesia and for sedation in the intensive care unit [6].

Propofol decreases cerebral oxygen consumption, reduces intracranial pressure and has potent anticonvulsant properties. In addition, propofol contains a phenolic hydroxyl group that donates electrons to free radicals and therefore may complement endogenous antioxidants[7]. The antioxidant characteristics of propofol may provide protection against ischemia of the central nervous system [8, 9, 10]. Propofol may restore excitatory amino acid transport after peroxideinduced oxidative stress [11].

Propofol Inhibited bronchoconstraction[12,13].and caused a decrease in arterial blood pressure that is partly due to a Vasodilation[14,15,16]. Although several mechanism of these Effects have been proposed,a decrese in intracellular calicium[Ca^{2+}],has been suggested to be a possible mechanism.In fact,propofol reduced [Ca^{2+}],,and Inositol 1,4,5-triphosphate (ip_3) in Various cells including smooth muscle cells[17,18,19,20].

Endothelin-1 (ET-1) is a 21-amino acid peptide, synthesized primarily by the vascular endothelium, which produces a slowly-developing, sustained contraction [21]. In vascular smooth muscle, the ET-1 interaction at the ETA receptor elevates [Ca^{2+}]i due to both an initial release of Ca2+ from the intracellular stores and a prolonged entry of extracellular Ca2+ through L-type channels [22,23,]. ET-1 stimulates phospholipase C,

The formation of inositol 1, 4, 5-trisphosphate (Ins(1,4,5)P3), the generation of diacylglycerol and PKC activity[24,25]. (Ins (1, 4, 5) P3 is known to regulate Ca2+ release from the sarcoplasmic reticulum [26].

Endothelin produced by vascular endothelial cells is a potent vasoactive peptide that elicits prolonged contraction of vascular smooth muscle cells. The effects of propofol on endothelin-1-induced intracellular signaling in an aortic smooth muscle Propofol inhibited the endothelin-1-induced Ca^{2+} influx, but this was significant only at supura clinical concentrations. The endothelin-1-stimulated formation of inositol phosphates was significantly suppressed by propofol. However, propofol had no effect on the formation of inositol phosphates.. Propofol inhibited the endothelin-1-induced formation of choline. Propofol had no effect on the binding of endothelin-1 to its receptor. These results suggest that propofol inhibits endothelin-1-induced intracellular signaling in vascular smooth muscle cells.

Glibenclamide, an adenosinetriphosphate (ATP)-sensitive potassium (K^+)-channel inhibitor,antidiabetic sulfonylurea drug glibenclamide prevents the opening of these channels. Inact, the antidiabetic (insulin-releasing) properties of this drug are related to closing of the KATP- channels in the pancreatic islet cells (and subsequent depolarization of the cell membrane followed by elevation of intracellular calcium and, eventually, insulin release).

The aims of the present study were to determine the effects of an adenosine triphosphate–sensitive Potassium channel opener nicorandil may suppress or induced anesthetic action of Propofol, grip strength test in mice.

Materials and Methods

Grip Strength Test[27,28]

This test is used to evaluate muscular strength or neuromuscular function in rodents. Initially the animals are tested for their normal reactivity. They were exposed to a horizontal metallic wire 30 cm long suspended in air. Mice are allowed to hang with its fore limb and their ability to catch with their hind limbs within 5 seconds was included for the test. After administration of the drug the animals

not able to touch the wire with the hind limbs within 5 seconds or animals which fall off were considered to have decreased tone. The animals were injected with test drugs and their muscle tone evaluated by this test. Forelimb;- Before treatment Mice are allowed to hang with its fore limb and their ability to catch with their hind limbs within 5 seconds; Hind limb;- After administration of the drug the animals not able to touch the wire with the hind limbs within 5 seconds or animals which fall off were considered to have decreased tone

Animals

48 Swiss *Albino* mice were included in the study in 8 groups of 6 animals in each (n=6). Experiments were performed on either sex of Swiss albino mice (125–150g). Animals were procured from the animal house. Maintained on a natural day–night cycle (12hr dark: 12hrs light) at room temperature of about 24-26°C, with free access to standard food pellets and water. Animals were acclimatized for at least ten days before exposure to behavioral experiments. Experiments were carried out between 10:00-17:00 hours. The animals were obtained from central animal house of JKKMMRFs, Namakkal. All the experimental procedures and protocols were viewed and approved by the Institutional Animal Ethics Committee (IAEC) of the institute,

Chemicals & drugs

All standard chemicals used in this study were of analytical grade. Methohexital for injection, (indiamart New Delhi,india) Propofol Taj Pharmaceuticals Ltd. Maharashtra, India), Nicorandil nicorandil was prepared from a 100 mM stock solution (in DMSO), diluted in 10% polyethoxylated castor oil to a final concentration of 5 mM. Sun pharmaceuticals (No. 303RI82344; Maharashtra, India), Endothelin-1 (Tocris Bioscience. New Delhi,india**)** Endothelin-1 Soluble to 1 mg/ml in water, glibenclamide ((Prudence Pharma Chem**.** Gujarat, India.**)** glibenclamide Soluble in 3% Tween 80. Glibenclamide was dissolved in 0.1 N NaOH, and pH was adjusted to 7.6 with 0.1 N HCl.[29]

Grouping: Groups are divided as follows,

Groups	Treatment Group
Group I	Solvent control (Sodium chloride alone (0.9%),
Group II	Referance control (Methohexital 40 mg/kg i.v) [30].
Group III	Test dose- (Propofol 12-26 mg/kg (i.v) [30].
Group IV	Pretreated with ATP Sensitive potassium channels opener With Test dose (Nicorandil, 10mg/kg µmol (i.p)[31].+ (Propofol 12-26mg/kg (i.v).
Group V	Pretreated with PLC inhibitor With Test dose (Endothelin-1 (0.1-100 ng; 0.04-40 pmol/kg intradermal injection) [32]. + (Propofol12-26 mg/kg (i.v)
Group VI	Pretreated with PLC inhibitor With ATP Sensitive potassium channels opener (Endothelin-1 0.1-100 ng; 0.04-40 pmol/kg intradermal injection) + Nicorandil, 10mg/kg µmol (i.p)
Group VII	Pretreated with ATP Sensitive potassium channels opener, PLC inhibitor With Test dose. (Nicorandil, 10mg/kg µmol (i.p .+ (Endothelin-1 0.1-100 ng; 0.04-40 pmol/kg intradermal)+ (Propofol12-26mg/kg i.v)
Group VIII	Pretreated with potassium channel blocker With Test dose. glibenclamide (0.5 g/kg i.p.) [33]. + (Propofol 12-26mg/kg i.v)

RESULTS

Objective of the study on effects of an adenosine triphosphate–sensitive Potassium channel opener nicorandil may suppress or induced anesthetic action of Propofol, grip strength test in mice. Grip Strength Measurement Muscle strength in mice was measured using the Animal Grip Strength System.This system uses an electronic digital force gauge that measures the peak force exerted upon by the action of the mice. Forelimb grip strength was measured.

Table 1: Grip Strength Measurement Muscle strength in mice fore limb-within 5 sec:-

Grip Strength test Fore limb(within 5 sec)		
Group	**Mean**	**SEM**
Group I	19.1	1.59
Group II	3.7	0.5
Group III	51.2	3.2
Group IV	86.6	6.2
Group V	105.4	4.34
Group VI	72.2	8.28
Group VII	45.2	2.52
Group VIII	29.06	4.8

Table 2: Grip Strength Measurement Muscle strength in mice Hind limb-within 5 sec:-

Grip Strength test Hind limb(within 5 sec)		
Group	Mean	SEM
Group I	16.6	2.44
Group II	4.3	1.5
Group III	49.6	6.4
Group IV	74.8	4
Group V	95.2	2.62
Group VI	66.4	6.4
Group VII	40.6	4.72
Group VIII	31.18	5.6

Table 3: different drug groups Grip Strength Measurement Mean ± SEM

Group	Drug Name	Dose (mg/kg)	Grip Strength test	
			Forelimb (within 5 sec)	Hindlimb (within 5 sec)
			Mean±SEM	Mean±SEM
Group I	Sodium chloride (solvent control)	0.9% Nacl	19.1 ± 1.59	16.6 ± 2.44
Group II	Methohexital (Referance control)	40 mg/kg mg/kg (i.p)	3.7± 0.5	4.3± 1.5
Group III	Propofol (Test dose)	(12-26 mg/kg (i.v)	51.2± 3.2*	49.6± 6.4*
Group IV	Pretreatedwith Nicorandil, + Propofol	(10mg/kg µmol (i.p)	105.4± 4.34***	95.2± 2.62***
Group V	Pretreated Nicorandil, with Endothelin-1	(0.1-100 ng; 0.04- 40 pmol/kg)	86.6± 6.2**	74.8± 4.0**
Group VI	Pretreated with Endothelin-1 + Propofol	12-26 mg/kg (i.v)	72.2± 8.28	66.4± 6.40
Group VII	Pretreated with Nicorandil, Endothelin-1 + Propofol	10mg/kg µmol (i.p)	45.20± 2.52*	40.60± 4.72*
Group VIII	Pretreated with glibenclamide + Propofol	(0.5 g/kg i.p.)+(12-26 mg/kg (i.v)	29.06 ± 4.8*	31.18 ± 5.6*

Statistical analysis of parametric data (induction and recovery times) was performed using one-way analysis of variance (ANOVA) Asterisks (**) denote the significant level P values=p<0.01. (***) Extremely significant p<0.001. A repeated measure analysis of variance with time and treatment as factor was used to compare latency. All results are expressed as mean ±SEM and differences were considered significant at Group III (*) P<0.05 significant, , Group IV-*** P<0.001, Group V--** P<0.01, Group VI--** P<0.01, Group V II--* P<0.05, Group VIII--* P<0.05 Statistical analysis of parametric data (Muscle strength of Forelimb, Hindlimb) was performed using one-way analysis of variance (ANOVA).

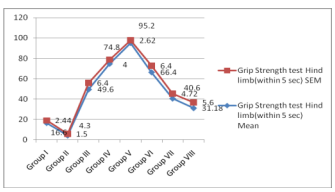

Fig.1 Effects of administration of Propofol with Adenosine triphosphate sensitive potassium (K_ ATP) channel opener. On Grip Strength MeasurementRepresents the mean ± SEM of group (n = 6).

DISCUSSION

The aims of the present study were to determine the effects of an adenosine triphosphate–sensitive Potassium channel opener nicorandil may suppress or induced anesthetic action of Propofol, grip strength test in mice.

The following observation can be drawn based on the 8 groups of mice results of the studies:

The effect of Adenosine triphosphate–sensitive Potassium channel opener nicorandil [34, 35] with propofol on muscle function the present study demonstrates that significantly Enhanced muscle function, as evidenced by increased grip strength, as well as Decreased fatigue. Improve the physical function. P<0.001*** (Forelimb, 105.4± 4.34, Hindlimb, 49.6± 6.4). Ref.control vs potassium channel opener.

The function of KATP-channels is modulated by the metabolic state of the cells. It is especially important that opening occurs in response to a decrease in intracellular ATP levels and tissue Opening of these channels characteristically results in potassium efflux, cellular hyperpolarization, and reduction of the entry of extracellular calcium via voltage dependent calcium channels, and ultimately, vascular smooth muscle relaxation.

Glibenclamide altered the neuromuscular strength in mice, they significantly. P<0.05* (Forelimb, 29.06 ± 4.8, Hindlimb, 31.18 ± 5.6) decreased the muscle strength when administered concomitantly.(Ref. control vs Test dose). glibenclamide completely blocks the muscle function.[Suzuki] It has also been reported that glibenclamide acts as a vasorelaxant by stimulating the release of nitric oxide (NO)from the endothelium.

There were no significant alternations in motor coordination with propofol alone treated with mice. P<0.05* (Forelimb, 51.2±3.2, sec, Hindlimb, 49.6±6.4).

Whereas pretreatment of PLC inhibitor The effects of propofol with Nicorandil on the endothelin-1-induced activation of phosphatidylinositol-hydrolyzing phospholipase C and phosphatidylcholine-hydrolyzing phospholipase Potassium channel opener nicorandil Partialy inhibition the muscle strength the grip strength compared with propofol alone. P<0.01** (Forelimb,86.6± 6.2, Hindlimb 74.8± 4.0) Ref.control vs Testdose.

Grip strength of the mice Propofol alone treated was significantly inhibitied. Grip strength test showed increase of (a) forelimb and (b) hindlimb grip strength in the mice treated with the nicorandil with propofol.A decline in muscle strength has been reported potassium channel blocker with propofol.

CONCLUSION

In conclusion, our study showed that in the Adenosine triphosphate sensitive potassium channels are present in a wide variety of tissues and are believed to link cellular metabolic status and excitability in vascular smooth muscle cell KATPchannels opener Nicorandil proved intravenous anesthetics propofol Enhaned neuromuscular strength in mice.

The effects of propofol on the endothelin-1-induced activation of muscle function. KATPchannels regulate the membrane potential, which controls calcium entry through voltage-dependent calcium channels, and thereby contractility through changes in intracellular calcium. Present study has suggested that opening KATPchannels in vascular smooth muscle causes vasodilation;

REFERENCES:-

1. IONA Study Group (2005) Determinants of coronary events in patients withstable angina: results from the impact of nicorandil in angina study. Am Heart J150: 689.
2. Das B, Sarkar C (2003) Mitochondrial K ATP channel activation is important in the antiarrhythmic and cardioprotective effects of non-hypotensive doses of nicorandil and cromakalim during ischemia/reperfusion: a study in an intact anesthetized rabbit model. Pharmacol Res 47: 447–461.
3. Narita M, Takamori K, Kawashima N, Funada M, Kamei J, Suzuki T, Misawa M, Nagase H. Activation of central ATPsensitive potassium channels produces the antinociception and spinal noradrenaline turnover-enhancing effect in mice. Psychopharmacology (Berl) 1993;113:11–4
4. Lohmann AB, Welch SP. ATP-gated K(_) channel openers enhance opioid antinociception: indirect evidence for the release of endogenous opioid peptides. Eur J Pharmacol 1999;385:119–27
5. Ocana M, Cendan CM, Cobos EJ, Entrena JM, Baeyens JM.Potassium channels and pain: present realities and future opportunities.Eur J Pharmacol 2004;500:203–19
6. Bryson HM, Fulton BR, Faulds D: Propofol. An update of its use in anaesthesia and conscious sedation. Drugs 50: 513-59, 19
7. Murphy PG, Myers DS, Davies MJ, Webster NR, Jones JG: The antioxidant potential of propofol (2,6-diisopropylphenol). Br J Anaesth 68: 613-618, 1992
8. Boland A, Delapierre D, Mossay D, and Hans P, Dresse A: Propofol protects cultured brain cells from iron ion-induced death: Comparison with trolox. Eur J Pharmacol 404: 21– 27, 2000
9. Ito H., Watanabe Y, Isshiki A., Uchino H: Neuroprotective properties of propofol and midazolam, but not pentobarbital, on neuronal damage induced by forebrain ischemia, based on the GABAA receptors. Acta Anaesthesiol Scand. 43, 153–162, 1999
10. Ozturk E, Demirbilek S, But AK, Saricicek V, Gulec M, Akyol O, Ersoy MO: Antioxidant properties of propofol and erythropoietin after closed head injury in rats. Neuro- Psychopharmacology & Biological Psychiatry 29: 922–927, 2005
11. Sitar S, Hanifi-Moghaddam P, Gelb A, Cechetto D, Siushansian R, Wilson J: Propofol prevents peroxide-induced inhibition of glutamate transport in cultured astrocytes. Anesthesiology 90:1446-1453, 1999
12. Clgarini I,Bannet F,Lorino AM,Harf A;Comparison of the effects of Fentanyl on respiratory mechanism under propofol of thiopental anesthesia.Acta Anesthesia Scand 1990;253-6
13. Pederson CM; The effect of sedation with propofol on postoperative bronchoconstriction in patients with hyperreactive airway disease,Intensive care Med 1992;18;45-6
14. Bently GN,Gent JP,Goodchild cs;Vascular effects of propofol;smooth muscle relaxation in isolated veins and arteries.J Pharmacol 1989;41;797-8.
15. Park WK,Lynch CI,Johns RA;Effects of propofol and thiopental in isolated ret aorta and pulmonary artery.Anesthesiology 1992;77;956-63
16. Gelb AW,ZhangC,Hamilton JT; Propofol induces dilation and inhibits constriction in guinea pig basilar arteries,Anesth Analg 1996;83;472-6
17. Xuan YT,Glass PS;Propofol regulation of calcium entry pathway in cultured a10 and rat aortic smooth muscle cells.Br.J Pharmacol 1996;117;5-12
18. YamakaseM,Hishmann CA,Croxton TL;Inhibitory effects of thiopental, ketamin, and propofol on voltage dependent Ca2+ channels in porcine brachial smooth cells.Anesthesiology 1995;83;1274-82
19. Samain E,Bouillier,Marty J,Safar M,Dagher G;The effect of propofol on angiotension II- Induced Ca2+Mobilization in aortic smooth muscle cells from normotensive and hypertensive rets,Anesth Analg 2000;90;546-52
20. Tanabc K,Koazwa O,Kaida T,MatsunoH,Niwa M,et,al;Inhibitory effects of propofol on intracelluar signaling by endothelin-1 in aortic smooth muscle cells,Anesthesiology 1998;88;452-60
21. Yanagisawa,Anagisawa,M., Kurihara H., Kilmura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K. & Masaki, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature, 332, 411-415.
22 Goto, K., Kauya, Y., Matsuki, N., T Ca2+akuma, Y., Kurihara, H., Ishikawa, T., Kimura, S., Yanagisawa, M. & Masaki, T. (1989). Endothelin activates the dihydropyridine- sensitive, voltage-dependent Ca2 + channel in vascular smooth muscle.Proc. Natl. Acad. Sci. U.S.A., 86, 3915-3918.
23. Xuan, Y.T., Watkins, W.D. & Whorton, A.R. (1991). Regulation of endothelin-mediated calcium mobilization in vascular smooth muscle cells by isoproterenol. Am. J. Physiol., 260, C492- C502.
24 Renterghem, C.V., Vignel, P., Barhanin, J., Schmid-Alliana, A., Frelin, C. & Lazdunski, M. (1988).Molecular mechanism of action of the vasoconstrictor peptide endothelin. Biochem. Biophys. Res. Commun., 157, 977-985.
25. Xuan, Y.T, Whorton, A.R & Watkins, W.D (1989). Inhibition by nicardipine of endothelin-mediated inositol phosphate formation and Ca2+ mobilization in smooth muscle cells. Biochem. Biophys. Res. Commun., 160, 758-764.
26. Berridge, M.J. & Irvine, R.F. (1989). Inositol phosphates and cell signaling. Nature, 341, 197-205.
27. Anesthesia and analgesia in laboratory animals. Kohn DF, Wixson SK, White WJ, Benson GJ (eds.) AcademicPress.SanDiego.1997.
28. Laboratory animal anesthesia. Flecknell PF. Academic Press. London. 1996.
29. Suzuki T, Sekikawa T, Nemoto T, Moriya H, Nakaya H. Effects ofnicorandil on the recovery of reflex potentials after spinal cordischaemia in cats. Br J Pharmacol. 1995; 116:1815-20.
30. Gaertner, DJ, TM Hallman, FC Hankenson, MA Batchelder. 2008. Anesthesia and Analgesia in Rodents. Anesthesia and Analgesia in Laboratory Animals. Second Edition, Academic Press, CA.
31. Attolini L, Bruguerolle B. Kinetics of bupivacaine after nicorandil treatment in mice. J .Pharm Pharmacol. 1996 Jul; 48(7):749-52.
32. Servitja et al (1998) Involvement of ETA and ETB receptors in the activation of phospholipase D by endothelins in cultured rat cortical astrocytes. Br.J.Pharmacol. 124, 1728. PMID: 9756390.
33. Ashcroft FM, Gribble FM. ATP-sensitive K+ channels and insulin secretion: their role in health and disease. Diabetologia1999; 42:903–919
34. Taira N. Nicorandil as a hybrid between nitrates and potassium channel activators. Am J Cardiol. 1989; 63:18J-24J.
35. Sakai K, Akima M, Kamachi S, Moriyasu M, Kitajima S.Interrelationship of cardiovascular effects, plasma levels of nicorandil, and vascular cGMP formation in conscious rats. J Pharm Pharmacol.1998; 50:661-6.