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Research Article

ANTINOCICEPTIVE EFFECT OF PENTAZOCINE IN DIABETIC RATS

K.S. Sridevi Sangeetha[*1], S. Umamaheswari[1], T.S.Subashini[2]
[1]Department of Pharmacology, Faculty of Pharmacy, Sri Ramachandra University, Porur, India.
[2]Department of Pharmacology, SRM Dental College, Ramapuram, Chennai, India.
Email: sangeethsb@gmail.com

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Abstract

Aim: The objective of the current study is to investigate the antinoceptive effect of Pentazocineon streptozotocin-induced diabetic rats.

Methods: Diabetes mellitus was induced by using streptozotocin, dose of 50mg/kg body weight through lateral tail vein injection. After 48 hours, rat with blood glucose range of 200–300mg were used for experiment. NPH – insulin was given by subcutaneous injection at the doses ranging from 3-5 units daily from 34th day and continued till 41st days. The analagesic activity was measured using tail flick method.

Result: Graph was plotted between the tail flick response in seconds and days.

Conclusion: From the result, it was concluded that the analgesic effect of pentazocine in insulin treated diabetic rat were more than in normal rat, eventhough the glucose levels were almost the same.

Keywords: Antinociceptive, Diabetes, Pentazocine, Streptozotocin.

Introduction

Diabetes mellitus is a common disorder affecting majority of population in the world. Apart from the hyperglycemia, the majority of the symptoms of diabetes mellitus are attributed to its secondary effects. As known diabetes mellitus causes retinopathy, atherosclerosis, hypertension, changes in central nervous system activity, which often may be lethal if not treated for the underlying disorder.

It is a known fact that wound healing is markedly reduced during diabetes mellitus. Many factors are implicated for the above, changes in immune response, changes in lipid metabolism and changes in the function of collagen. As wound healing is affected by diabetes mellitus, it might interfere with mediators of wound healing, including those for inflammation. Increase in wound healing time caused by analgesics and antipyretic necessitates their use for a

longer period of time. Hence the changes in analgesia during diabetes mellitus might be an important topic of study to enables the selection of a good analgesic which has high efficiency, high potency and shows less incidence of adverse reactions, thus not burdening, the already increased time to wound healing.From the survey it is found that diabetes mellitus increases antinociception and also varied effect on different analgesic, hence due to the contradictory report it was thought that the present study more light on antinociception, choice of suitable analgesic, doses and their adverse effect during diabetes mellitus.

Diabetes Mellitus is associated with high blood glucose levels. This hyperglycemic state is due to the diminished secretion of insulin from the β-pancreatic cells. This diminished secretion may be associated with damage or destruction of β-pancreatic cells. Diabetes Mellitus is of two types viz., insulin dependent Diabetes Mellitus (IDDM) and Non-insulin dependent Diabetes Mellitus (NIDDM).

Apart from the pathological causes of diabetes mellitus, it is induced experimentally by using diabetogenic agents like Streptozotocin and Alloxan. Besides primary complications like hyperglycemia, secondary complications like cataract[1] and retinopathy[2] are also observed. Cataract is associated with increase or decrease in concentration of copper and Copper containing enzyme-superoxide dismutase, while retinopathy is due to lipid peroxidation.Diabetes mellitus also effects the central nervous system. It causes changes in both barrier and transport functions of the cerebral microvessels[3]. Apart from causing changes in blood-brain barrier, it inhibits the brain synaptic plasma membrane Calcium ATP – ase[4] in cells from several tissues. In Diabetes mellitus, dysfunction of ATP-sensitive Potassium channel[5] produces poor antinociceptive effect to mu-opioid agonists. This poor antinociceptive effect affects selectively supraspinal mu-1 opioid receptor and not spinal mu-2 opioid receptor[6]. But, it will not affect the delta and kappa opioid receptor agonists[7]. The high levels of Potassium ions in diabetic rats cause the release of SPL 1 from the spinal cord[8]. Diabetes mellitus also causes behavioural changes, which occur at the brain opioid system[9].

Profile of Streptozotocin:

Streptozotocin is an antineoplastic antibiotic produced by the growth of a *Streptomuycesachromogenes* variant or by synthesis. It is used mainly in the treatment of pancreatic (islet - cell) tumors[10].The streptozotocin induced diabetes mellitus has advantages over alloxan because only streptozotocin liver microsomes exhibited changes in the cytochrome P-450 normal spectral characteristics. In rats, diabetes was induced by using intravenous dosage of 50 mg/kg body weight (using 2% W/v solutions in saline buffered with dextrose solution at pH-5)[11].

Streptozotocin is a white or yellow coloured powder having molecular formula $C_8H_{15}N_3O_7$and molecular weight of 265.2. It is stable when frozen and protected from moisture and air. Its solution has maximum stability at pH-4 and stability decreases rapidly at higher or lower pH. The solution should be prepared just before use and upon standing they takes on a yellow to brown colour and effervescence, indicating decomposition[12].

Pentazocine

Pentazocine is an opioid analgesic acting on the central nervous system. It exhibits strong analgesic activity. The antinociceptive effect of pentazocine was found to be greater in diabetic mice than the non-diabetic mice[13]. This enhanced antinociceptive action may be due to its action on kappa opioid receptor and hyporesponsiveness action of mu-opioid receptor. It also exhibits antitussive effect by acting on the delta opioid receptors in mice but, in non diabetic mice it is medicated through both mu and kappa opioid receptors[14].

Due to its action on mu, kappa and delta receptors, it was preferred and its effects on diabetes were also varied, hence pentazocine was chosenfor the study.

Materials and Methods

Animals

Wistar albino rats of either sex, weighing 130-180g, purchased from king institute, Guindy were used for the experiment. The animals were fed with standard rodent pellets (Gulmohar, Hindustan Liver Limited) and water 'ad' Libitum. Animas were divided into four groups each containing 7 rats.

Diabetes inducing agent- Preparation of streptozotocin injection.

The intravenous injection of streptozotocin was prepared by using 2% w/v solution of drug in saline buffered with citrate dextrose injection at PH-5.

Preparation of Citrate Dextrose Buffer[15]

Solution A - 2.2 g of sodium citrate, 0.73 g of anhydrous citric acid and 2.4g of dextrose monohydrate were weighed and dissolved in 25ml of water for injection. The final volume was made to 100ml with water for injection.

Solution B - 1.32 g of sodium citrate, 0.44g of anhydrous citric acid, 1.47g of dextrose monohydrate were dissolved in 25ml of water for injection. The final volume was made upto 100ml with water for injection.

15ml of solution A or 25ml of solution B were mixed and required pH was confirmed by using pH meter.

Preparation of O–Toluidine reagent.

2.4g of boric acid and 2.5g of thiourea was dissolved in 100ml of solution containing distilled water, glacial acetic and freshly distilled O-toluidine in the ratio of 10:75:15 v/v.

Insulin -NPH – Insulin was used at the dose of 3-5 units given by subcutaneously.

Opioid Analgesic –Pentazocine: It was given intraperitoneallydose of 30mg/kg body weight was used.

Experimental Groups

Four groups each of seven rats were used for the experiment

Group I - Control

Group II - Control, Treated with pentazocine after 40th and 41st day.

Group III - Non insulin treated diabetic rats, treated with pentozocineafter 40th and 41st day.

Group IV - insulin treated diabetic rats, insulin treatment started after 33rd day and pentozocine treatment after 40th and 41st day.

Induction of Diabetes Mellitus In Rats

Diabetes mellitus was induced by using streptozotocin, dose of 50mg/kg body weight throughlateral tail vein injection[16]. After 48 hours, rats with moderate diabetes having glucosuria and hyperglycemia with blood glucose range of 200–300mg/100ml were used for experiment.

Estimation of Blood Glucose Level

Blood was collected from the orbital plexus[17] through capillary tube and immediately transferred to the test tube containing Ethylene Diamine Tetra Aceticacid (EDTA). Serum was separated after centrifuging with mixture of 0.1ml of blood and 3ml of 10% trichloro acetic acid.

Method of Estimation

Fasting Blood glucose was estimated by O-toluidine method[18].

1 ml of serum was mixed with 4 ml f the O-toluidine reagent. Standard glucose solution containing 25-100mg and the blank containing 1ml of distilled water were also processed in the same manner and heated in the water bath for 8 minutes. Transmittance was adjusted to 100% with the blank at 630nm. Then, the standard and test readings were noted and the concentration of glucose in the test sample was calculated by using the standard readings.

Treatment with Insulin:

Apart from hypoglycaemic action, insulin also has antinociceptive effect[19]. In our study, we are concerned with the algesic and analgesic activities. So, the effects of insulin on diabetes mellitus and on the opioid analgesic treated rats were studied.NPH – insulin was given by subcutaneous injection at the doses ranging from 3-5 units daily[20]

according to the blood glucose level to the group IV. This treatment was started 33 days after streptozotocin injection, because the hypoalgesic response was attained only after 4 weeks[21].

Study of Algesic Response and Analgesic Effect: In diabetic stage, there is significant in pain threshold and this elevation of pain threshold achieve peak level, four weeks after streptozotocin induction. This hypoalgesic state was thought to be mediated by opioid receptors (mu and delta receptors)[22]. So, opioid analgesic werechoosen for their effects in the diabetes. Pentozocine was choosenbecause it acts on mu, kappa and delta receptors and its nociceptive effect was greater in diabetic mice than nondiabetic mice. Also, in streptozotocine induced diabetes mellitus there was an alteration in mu-opioid receptor to its agonist, so the drug had wide range of activity on its receptor.

It was given intraperitionially at the dose of 30mg/ kg body weight to the group II,III& IV on 40th and 41st day after streptozotocine administration; because hypoalgesic response occurred only after 4 – 6 weeks.

Study of Analgesic Activity[23]

The analgesic activity was tested in rats by the "Rat Tail Flick Method" using the "INCO ANALGESIOMETER". Group II, III & IV animals were used for the study. The tail withdrawal reaction time in seconds was recorded one hour after pentazocine administration and results were tabulated.

Statistical Analysis: All the grouped data were calculated statistically and the significance of various treatment group was calculated using Student's t-test and the results were expressed as mean ± SEM.

Results: There was decrease in algesia and increase in threshold of analgesia seen from the Figure 1. This increase in analgesic effects was statistically significant in group III and group IV. The analgesic effects increased due to hyperglycemia decreased in correlation with decrease in glucose levels with insulin treatment in group IV. The analgesic effects of the opioid agonist pentazocine was increased significantly with hyperglycemia as seen in group III.

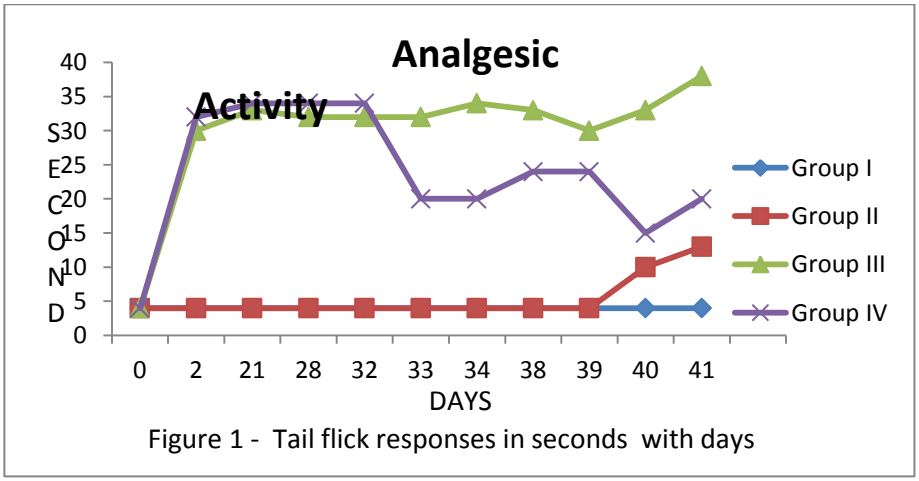

Figure 1 - Tail flick responses in seconds with days

Treatment with insulin increased the effects of pentozocine significantly but not as significant as group III. The increase in analgesic effect was almost 4 times in group III and almost double in group IV when compared with group II. Although the glucose levels of group IV with insulin treatment was to the control levels of group II the analgesic effects were elevated in group IV, when compared to group II. The algesic effect was increasing with increase in glucose levels and decreased with treatment with insulin.

Discussion

The antincociceptive effect was found to be increasing in hyperglycemia as observed from the results. There are a number of factors which might be the cause to the above effect. Certain factors derived from spleen, mononuclear cell are implicated for reduced opioid receptor mediated analgesia.Stimulation of periaqueductal gray matter during diabetes mellitus may also account for anlagesia[24]. The changes seen in the opioid receptor may be due to its effects on K^+ channel.

Opioid analgesics acting on its receptor causes decrease in levels of Ca^{2+} and increase in K^+. In diabetes the metabolic changes causes decrease the opening up of ATP – are sensitive K^+ channels which causes analgesia, and also opens up the K^+ channel which increasesin the release of substance P.

Diabetes mellitus has got varied effects on the opioid analgesics[25]. It decreases the analgesic activity of morphine but from the results it was clear that the analgesic effect of pentazocine was increased. These variation might be due to the differences in sensitivity of opioid receptor to hyperglycemia of diabetes mellitus. In streptozotocine induced diabetes mellitus the hyporesponsiveness of opioid receptor was associated with mu-receptor and not delta and kappa receptors. This might be the reason why the analgesic activity of pentazocine was increased in diabetes mellitus while that of morphine was decreased. Moreover the effects on mu-receptor was due to mu-1 receptor and not through mu-2 receptor.Diabetes also alter the opioid binding to mu and kappa receptor in the rat brain which might also account for the difference observed for analgesia with morphine and pentazocine.

Changes in calcium might also account for the changes in analgesic effect. When calcium channel blockers were given, there was an increase in analgesia, with morphine which was then reduced in streptozotocin induced diabetes mellitus, these effects were reversed by Calcium Channel agonist[26].

Eventhough treatment with insulin lowered the glucose level to normal,the analgesic effect was still abnormal when compared to the control group as seen from results. The antinociceptive effect was more in insulin treated ratsthan in the control. The analgesic effect of the Pentazocine was still significantly higher in insulin treated diabetic rats

when compared with the control, even though the glucose level were almost the same. This might be due to the analgesic effects of insulin growth factor (IGF)[27] released along with growth hormone (GH) and it is known that growth hormone and insulin are secreted together.

Conclusion: Itis clear from the study that antinociception increase and the analgesic effect of Pentazocine was also increased and insulin had effect on antinociception as treated animal with normal glucose level showed varied responses in diabetes mellitus, during treatment. It was also clear from the review and from the present study that pentazocine was more efficient analgesic than morphine in diabetes mellitus, and hence it can be the preferred analgesic in those conditions.

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Corresponding Author:
K.S. Sridevi Sangeetha[*1],
Email: *sangeethsb@gmail.com*