

A STUDY ON THE POSSIBLE ASSOCIATION BETWEEN GLYCEMIC STATES AND 7-HYDROXYFLAVONE INDUCED ANALGESIA

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Summary

Previous studies in chemical induced diabetic animal models showed varied results on the association between blood glucose and pain threshold. In the present modified approach the changes in blood glucose level were induced by exogenous dextrose administration (hyperglycemia, 2mg/kg;i.p) or forced swimming in water at room temperature (28-30⁰C) for 30 minutes (hypoglycemia) in mice and pain threshold measured by abdominal constriction assay using 7-hydroxyflavone. Blood glucose was measured using Ames glucometer. The results suggest that the pain threshold is independent of blood glucose in acute conditions. However extrapolation of the data to chronic conditions is debatable.

Key words: swim stress, dextrose, hypoglycemia, hyperglycemia, blood glucose, antinociception.

Introduction

Diabetic neuropathy is a painful condition experienced in diabetes mellitus (DM) and control of DM with the use of antidiabetic drugs alleviates neuropathic pain. This lead to belief that there exists an association between blood glucose and pain threshold. Experimental studies

were conducted to examine this hypothetical association in streptozotocin or alloxan induced diabetic animal model and however varied results were reported. It has been documented that streptozotocin or alloxan produces neuronal degeneration, changes in neurotransmitter function (1, 2), modification of metabolic profile of arachidonic acid (3), and reduction in the circulating endogenous opioid peptides (2). These factors may be responsible for varied results on the association between blood glucose and pain threshold in experimental diabetic animal model. In the present study a different approach was employed to study the association between blood glucose and pain threshold. Changes in blood glucose level in mice were induced by physiological manoeuvres such as exogenous dextrose administration (hyperglycemia) or forced swimming in water at room temperature (25-30⁰C) for 3 min, rather than by chemical agents. Earlier studies have identified the potent antinociceptive and anti-inflammatory properties of several flavone derivatives (4, 5, 6). In this study, 7-hydroxy flavone (7-HF), a flavone derivative and an opioid compound was used as antinociceptive agent and the antinociceptive response was assessed by abdominal constriction assay (7). Besides, 7-HF has been reported having significant effect on the blood glucose level (8) and hence assumes significance in selection of this compound for the present study.

Materials and Methods

I. Animals

Adult male albino mice weighing between 20-25 g were used in this study. They were housed under normal laboratory conditions with 12:12 light: dark cycle and had free access to food and water until the initiation of the experiment. The experiment was done during the light period. This animal study was approved by Institutional Animal Ethics Committee at Madras Medical College, Chennai, India

II. Drugs and chemicals

7-HF (Herboragics, Chennai, India); dextrose IP (Indian Drugs and Pharmaceuticals Ltd, Hyderabad, India); carboxy methyl cellulose sodium salt (Glaxo Laboratories, Mumbai, India) and all other chemicals of analytical reagent grade. 7-HF was suspended in 1% carboxy methyl cellulose in saline. Dextrose was dissolved in saline.

III. Assessment of antinociception

Mice were injected 10 ml/kg of 0.6% freshly prepared acetic acid intraperitoneally and the number of abdominal constrictions following this injection for a period of 30 min was recorded. A significant reduction ($p < 0.05$) in the number of abdominal constrictions as compared to vehicle treated group was considered as antinociceptive response (7).

IV. Measurement of blood glucose

The blood glucose was measured using Ames glucometer with appropriate glucostix. A drop of blood was collected from the animal by cutting the tip of the tail. The blood glucose was measured before initiation of the experiment and just prior to acetic acid challenge. The results were expressed as percentage change considering the initial blood glucose value of that animal as 100%.

V. Induction of hypo-and hyperglycemia

Hypoglycemia was induced by allowing the animal to swim in water in a polypropylene container (15 cm high; water column 51x38x30 cm height) at room temperature for 3 min. At the end of swimming mice were taken out of water, wiped gently with a clean dry towel and subjected to antinociceptive assay. Blood glucose was measured as described earlier. Hyperglycemia was induced by injecting dextrose 2g/kg i.p (dose selected based on pilot studies) 15 min prior to swimming or acetic acid challenge.

VI. Drug Treatment

The effective dose of 7-hydroxy flavone 100mg/kg (s.c) was selected based on earlier study. Animals were divided into six groups each group consisting of six animals and received the following treatment schedule. Group 1 : Vehicle control; Group 2: 7-hydroxy flavone 100 mg/kg; s.c administered 60 min prior to acetic acid challenge; Group 3: swim stress for 3 min just prior to acetic acid challenge; Group 4: Dextrose 2g/kg; i.p. administered 15 min prior to acetic acid challenge; Group 5: 7-hydroxy flavone 100mg/kg; s.c. followed by swim stress at 60 min just prior to acetic acid challenge; Group 6: 7-hydroxy flavone 100mg/kg; s.c. followed by dextrose 2g/kg; i.p. at 45 min and acetic acid challenge at 60 min. In all these groups the antinociception and blood glucose were measured as described earlier.

Statistical analysis

The data were analyzed statistically using ANOVA followed by Dunnett's 't' test. The value $p < 0.05$ was considered as significant response.

Results

Effect of 7-hydroxy flavone

7-hydroxy flavone (100 mg/kg; s.c.) produced a significant reduction in the number of abdominal constrictions as compared to vehicle treated mice ($p < 0.001$) and did not elicit significant change in the blood glucose level (Table 1).

Effect of swim stress

Swim stress produced a significant reduction in the blood glucose level (hypoglycemia, $p < 0.001$) and also in the number of abdominal constrictions ($p < 0.001$) when compared with vehicle treated mice (Table 1).

Effect of dextrose

Dextrose (2g/kg; i.p.) elevated the blood glucose level significantly ($p < 0.001$) and elicited a significant reduction in the number of abdominal constrictions ($p < 0.001$) as compared to vehicle treated mice (Table 1)

Effect of 7-hydroxy flavone on swim stress induced changes in blood glucose level and antinociception

7-hydroxy flavone (100mg/kg; s.c.) *per se* neither altered the blood glucose level nor modified significantly the hypoglycemia induced by swim stress. Rather, it enhanced the antinociceptive response induced by swim stress ($p < 0.001$) (Table 1).

Effect of 7-hydroxy flavone on induced changes in blood glucose level and antinociception

Dextrose (2g/kg; i.p.) *per se* produced a significant increase in blood glucose level as well as antinociception as compared to vehicle treated mice ($p < 0.001$). Conversely, 7-hydroxy flavone pretreatment attenuated the elevated blood glucose level ($p < 0.001$) without significantly altering the antinociception in the dextrose treated mice (Table 1).

Table 1. Effect of 7-hydroxy flavone (7-HF) on changes in blood glucose level and acetic acid induced abdominal constrictions induced by swim stress or dextrose

Treatment	¹ Blood glucose (%)	No. of abdominal Constrictions after 30 min
Vehicle	100.4 ± 5.6	60.7 ± 1.4
² 7-hydroxy flavones (7-HF)	103.3 ± 5.3	32.9 ± 0.4 ^a
³ Swim stress (SS)	65.3 ± 7.9b	19.6 ± 0.9 ^{a,b}
Dextrose	250.5 ± 16.5 ^{a,c}	13.8 ± 0.4 ^{a,c}
7-HF+SS	63.4 ± 4.8 ^a	4.7 ± 0.9 ^{a,d}
7-HF+Dextrose	161.7 ± 8.4 ^{a,e}	15.8 ± 1.3 ^a

Each value is the mean ± SEM of six experiments

¹Blood glucose was expressed as percentage considering the initial value as 100%

²7-HF100mg/kg; s.c was administered 60 min prior to acetic acid challenge

³Dextrose 2g/kg; i.p was administered 15 min prior to acetic acid challenge

^ap <0.001 Compared to vehicle treatment

^bp <0.001 Compared to 7-HF or dextrose treatment

^cp <0.001 Compared to 7-HF or Swim Stress

^dp <0.001 Compared to Swim Stress

^ep <0.001 Compared to dextrose treatment

Discussion

The results in the present study indicate that swim stress, dextrose and 7-HF produced hypoglycemia, hyperglycemia and euglycemia respectively. In all the three glycaemic states the antinociceptive response of 7-HF was observed. Interestingly, both hypo- and hyperglycaemic states potentiated the antinociceptive effect of 7-HF. While the swim stress induced hypoglycemia potentiated the antinociceptive effect of 7-HF, the dextrose-induced hyperglycemia did not elicit enhanced antinociceptive response of 7-HF. In contrast the dextrose-induced hyperglycemia was attenuated by 7-HF pretreatment. These findings suggest that the pain threshold may be independent of changes in the blood glucose level. However this view is in contrast to clinical symptoms in diabetic neuropathy wherein the elevated blood glucose level causes pain by decreasing the pain threshold. The differences in the relationship between blood glucose and pain threshold in the two situations, the one observed in the altered glycaemic states induced by physiological manoeuvres like exogenous dextrose administration or swim stress as in the present study and that observed in clinical diabetic neuropathy or in streptozotocin or alloxan induced diabetic animal model may be, in the former, the altered glycaemic state is reversible to normal without impairment to neuronal function thereby depicting true picture on pain threshold and in the latter, the irreversible glycaemic state (permanent hyperglycemia) leading to neuronal degeneration and the pain threshold measured in this condition might not be of true picture and so the varied results. Hence it can be conclusively stated that there exists no possible association between blood glucose and pain threshold in acute conditions and such an association is doubtful to exist in chronic conditions like DM with functional and structural changes at neuronal levels. Possibly, a hormonal factor may be insulin, not blood glucose, could participate in altering the pain threshold since it has been documented that insulin possesses inherent antinociceptive effect without affecting the glycaemic state (9) and that the antinociception is associated with elevated serum insulin level (10).

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