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2-DEOXY-D-GLUCOSE ALTERS THE CARDIO-RESPIRATORY PARAMETERS IN ANAESTHETIZED RATS

R. PANDEY, P. K. CHOUDHRY AND S. B. DESHPANDE*

*Department of Physiology,
Institute of Medical Sciences, Banaras Hindu University,
Varanasi – 221 005*

Abstract : 2-Deoxy-D-Glucose (2-DG), a synthetic analogue of glucose, is used as an anticancer agent either alone or in combination with other tumor treatment protocols. The present study was conducted to identify the systemic effects of 2-DG on parameters of vital importance. The blood pressure, ECG and respiratory excursions were recorded in anesthetized adult rats. At the end (after 120 min) of experiments, the plasma glucose and serum insulin levels were estimated. Injection of 2-DG (0.5 g/kg) produced an immediate increase in mean arterial pressure (MAP) and respiratory rate. The increase in MAP continued throughout the period of observation (120 min) and the maximal increase was seen at 90 min (27%). Whereas, the respiratory rate decreased by 17% at 15 min which decreased further to 37% by 120 min. Heart rate also decreased after 2-DG in a time-dependent manner and 40% decrease was observed at 120 min. Administration of 2-DG increased the plasma glucose level significantly (30%) as compared to saline control group but did not increase the serum insulin level. The results indicate that 2-DG alters the cardio-respiratory parameters by mechanisms unrelated to plasma insulin activity.

Key words : glucose analogue   anticancer-drugs
         cerebral ischemia/anoxia   hypoxia inducible factor 3α

INTRODUCTION

A non-metabolizable glucose analogue, 2-Deoxy-D-glucose (2-DG) inhibits glycolytic ATP production (1) and enhances the cytotoxic effects of anticancer agents or radiotherapy. Either alone or in combination with other anticancer therapeutic measures, 2-DG effectively suppresses the growth of tumor cells in animal models (2, 3) and a variety of human tumor cells (4, 5). The anti-viral effect of 2-DG has also been demonstrated against a number of viruses (6). It has also been used to produce calorie restriction in animal studies (7) and has been utilized to simulate conditions of glucose starvation (8). 2-DG competes with the glucose transporter and produces cytoglucopenia (9), therefore, alteration in serum insulin level is expected and such alterations may produce changes in the cardio-respiratory parameters. Since 2-DG

*Corresponding Author : Dr. S. B. Deshpande, Department of Physiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi – 221 005, India, Phone : 91-542-2369069; Fax : 91-542-2367568; E-mail : desh48@yahoo.com

interferes with the cellular ATP metabolism, it produces cellular anoxia, hence, respiratory changes are anticipated. The effects of 2-DG on cardio-respiratory parameters in reference to insulin level is not available despite its use in a variety of conditions mentioned above. Therefore, the present study was conducted to examine the respiratory and cardiovascular changes induced by 2-DG and their association with the glucose and insulin level was ascertained.

MATERIAL AND METHODS

Animals and anesthesia

All the experiments were performed according to the guidelines of the Ethical Committee of Institute of Medical Sciences, Banaras Hindu University, Varanasi, India for conducting animal experimentation. Male rats (200–250 g) belonging to Charles-Foster strain were used in this study. The animals were exposed to 12:12 h light/dark cycle. After fasting overnight with water *ad libitum*, the experiments were performed in the next morning (9.00 A.M.). The animals were anesthetized with an i.p. injection of urethane (1.5 g/kg).

Dissection and recordings

Dissection and recording procedures were similar to those described earlier (10, 11). Briefly, tracheal cannulation was done to keep the respiratory tract patent. Jugular vein cannulation was done to inject normal saline (0.9%) or 2-DG. Femoral artery cannulation was performed to record the blood pressure using a polyethylene cannula filled with freshly prepared heparinised saline (25 IU/ml of heparine from Biological Evans Ltd., Hyderabad, India). The cannula

was in turn connected to a Statham Strain Gauge pressure transducer (Biodevices, Ambala; India) to record BP. The pressure recordings were calibrated using mercury manometer and the mean arterial pressure (MAP) was taken as a parameter. The ECG was recorded on a chart recorder by connecting needle electrodes using limb lead II configuration (Right arm-Left leg). The respiratory excursions were recorded by connecting the force-displacement transducer with a thread secured to the skin over the xiphisternum. The animals were allowed to stabilize for 30 min after surgical procedures.

Experimental protocol

Animals were divided into two groups. In the first group, 0.5 g/kg of 2-DG (from Sigma Chemicals), in 1 ml saline was injected through the jugular vein and the recordings of blood pressure (BP), electrocardiogram (ECG) and respiration were taken continuously for the initial 5 min and then intermittently at 15 min interval up to 120 min. In the second group, equi-volume of saline (1 ml) used for injecting 2-DG was administered through the jugular vein and the recordings were taken as mentioned before.

Estimation of plasma glucose and serum insulin

The blood samples (~2 ml) were collected from the femoral artery after exposing the animals to 2-DG/saline for 120 min. Plasma glucose was estimated immediately by glucose oxidase method after collecting the samples. Subsequently, the remaining part of blood was allowed to clot and stored at 4°C up to 24 h. Serum thus separated was used for the estimation of insulin by enzyme linked immunoassay method using the rat

insulin ELISA kit. The procedures for insulin estimation were as per the instructions provided by the manufacturer (Mercodia Rat Insulin ELISA kit, Sweden).

Statistical analysis

All the data were presented as mean ± SEM. The time-response relation of 2-DG group was compared with control by using two-way ANOVA. The difference between glucose/insulin levels after 2-DG from the control group was compared by using Student's t-test for unpaired observations. A P<0.05 was considered significant.

RESULTS

2-DG altered the cardio-respiratory parameters

The resting MAP at the beginning of the experiment in 2-DG treated group (n = 3) was around 72 mm Hg. Injection of 2-DG (0.5 g/kg) increased the MAP by 14% within 2 min. It remained at that level for about 45 min. Thereafter, a progressive increase was

observed and at 120 min, there was about 30% increase in the MAP from initial (Fig. 1 and 2). These values at various time intervals are significantly different from the saline control group (P<0.05, two-way ANOVA).

After injection of 2-DG, there was no significant alteration in the heart rate within 2 min. But, subsequently it decreased in a time-dependent manner and the decrease was 40% of the initial at 120 min (Fig. 2).

The resting respiratory rate (RR) was 67/min. An immediate increase in RR (about 14%) occurred within 2 min after 2-DG administration. Thereafter, it decreased significantly (17%) within 15 min of injection. This level was maintained for about 90 min. Subsequently it decreased in a time-dependent manner and at 120 min, the decrease was about 37% of the initial value (Fig. 1 and 2).

In another group of experiments (n = 5), equal volume of saline that is used to
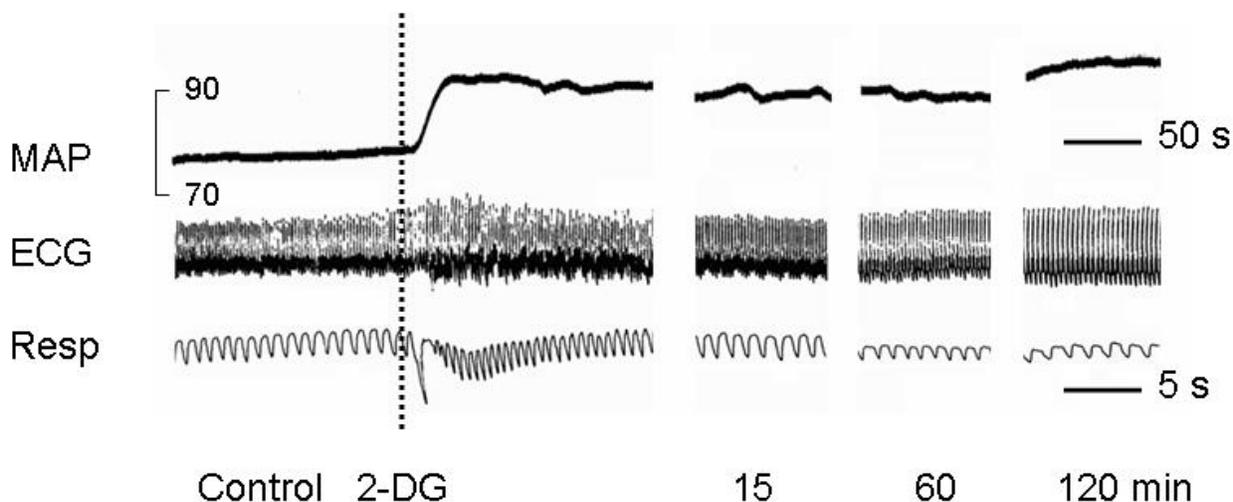
Fig. 1 : Actual recordings of an experiment showing the effect of 2-DG on cardio-respiratory parameters. Resp = Respiration. The dotted vertical line indicates the point of injection of 2-DG (0.5 g/kg).

dissolve 2-DG, was administered through the jugular vein. In this group, there was no alteration in the MAP, HR and RR up to the period of observation (120 min) and served as a time-matched control group (Fig. 2).

2-DG altered the plasma glucose level but not the serum insulin level

The data of plasma glucose and serum insulin are provided in Table I. In 2-DG treated group, the plasma glucose level increased significantly (P<0.05, Student's t test for unpaired observations) as compared to control rats. However, there was no increase in the serum insulin level in 2-DG treated rats (Table I).

TABLE I : Plasma glucose and serum insulin levels after saline or 2-DG. The values are mean±SEM from 5 experiments for saline and 3 for 2-DG treated group.

Parameters	Saline	2-DG
Glucose (mg/dL)	80.8±4.09	106.5±7.39*
Insulin (pmol/L)	90.3±29.46	79.9±24.73

An asterisk (*) indicates significant difference from saline control (P<0.05, Student's t test for unpaired observations).

DISCUSSION

In this study we evaluated the systemic effects of 2-DG in experimental animals. Our observations reveal that there was an immediate (within 2 min) increase in MAP and RR. Subsequently, MAP exhibited an increase while RR decreased drastically. The alterations in the cardio-respiratory parameters thus occurred in two different phases; an abrupt immediate change followed by delayed progressive changes. Further, our results indicate the increase in the plasma glucose level but not the serum insulin after 2-DG. Thus, our observations in comparison to earlier work (12) provide evidence for the increased MAP and decreased RR and HR unrelated to insulin levels.

2-DG competes with glucose transport and produces glucocytopenia and deficiency of cellular ATP (1). The immediate ventilatory changes produced by 2-DG indicate the possibility of chemoreceptor stimulation as indicated by the increased RR within 2 min. This is associated with simultaneous increase

Fig. 2 : 2-DG induced changes on cardio-respiratory parameters. The mean ± SEM values are obtained from 5 experiments for saline and 3 for 2-DG treated group. 2-DG or saline were injected at 0 time. An Asterisk (*) indicates significant difference from saline group (P<0.05, Students t test for unpaired observation, two way ANOVA).

in MAP. The chemoreceptor activation is known to increase the sympathetic activity leading to a pressor response. In a study elsewhere, i.v. administration of 2-DG even at lower concentrations (50 mg/kg) produced a rapid (about 2 min) increase in the discharge rate of adrenal nerve filaments (13). In another study, increase in cardio-vascular tone is also reported after systemic administration of 2-DG (14). These observations support for the immediate pressor response. Thus, the immediate effects of 2-DG on RR and MAP can be attributed to the chemoreceptor activation. Further, in a report elsewhere it is shown that 2-DG does not produce any alterations in blood PO_2, PCO_2 or pH (15). The mechanisms involved in the chemoreceptor stimulation by 2-DG thus appears to be independent of these stimulants. Recently, it is shown that 2-DG induces hypoxia inducible factor (HIF)-3α expression in a very short time (rapidly). HIF-3α participates in the oxygen homeostasis at cellular level and is expressed in various tissues (16). The immediate ventilatory response may be due to the effects produced by HIF-3α as suggested.

The increase in RR was followed by a decrease within 15 min of 2-DG administration and remained at that level up to 45 min. The decrease may be due to the CO_2 wash out brought about by increased RR. In that case, the RR should return to initial level after recovery or oscillate periodically. On the contrary, we observed time-dependent decrease in RR up to 120 min. Such decrease in RR may be due to the depression of vital respiratory centers. 2-DG induces energy deprivation and produces a generalized cerebral ischemia-like situation. It has been reported that cerebral ischemia stimulates the vasomotor area and increases

the blood pressure. This is associated with decreased heart rate and respiratory rate. These responses are known as Cushing's reflex (17). In the present study, we have observed similar changes in BP, HR and RR. Thus it is possible that 2-DG produces cerebral ischemia-like situation to bring about alterations in the cardio-respiratory parameters in delayed phase.

The cardio-respiratory changes can also be due to the increased insulin levels as reported elsewhere after 2-DG (8). On the contrary, insulin levels were not altered in the present study (Table I). The 2-DG induced HIF-3α expression is greater in the brain, lungs, liver and kidney as compared to that produced by insulin (16). Therefore, it appears that the cardio-respiratory parameters are not related to the insulin levels. The increase in glucose level may be due to the failure of glucose transport or due to the activation of adrenergic system. The evidences support the possibility of involvement of adrenergic system in raising blood glucose (8, 13).

The present observations provide information on the effects of 2-DG on the vital parameters in healthy anesthetized animals. The time-dependent alterations can not be due to the anaesthetic effect as there was no significant alteration in these parameters in time-matched control group (Fig. 1 and 2). Further, even within 120 min of observation period, there were significant alterations in the vital parameters as seen elsewhere in patients receiving 2-DG (17). In this clinical trial, the 2-DG treatment was withdrawn in 1/3rd of the cancer patients receiving 2-DG because of severe restlessness and increased blood pressure observed in these patients (17). Therefore,

the patients receiving 2-DG treatment require close monitoring of the vital parameters.

In conclusion, the results reveal two distinct phases of alterations in cardio-pulmonary parameters produced by 2-DG. The initial phase can be accounted for the peripheral chemoreceptor stimulation. The delayed phase can be due to the metabolic alterations produced by 2-DG. Our data signify the need for monitoring of cardio-respiratory parameters in patients receiving 2-DG treatment.

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