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Effects of metaraminol, insulin and trasylol on the responses to haemorrhage in dogs

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Summary

The effect of addition of metaraminol, insulin and trasylol to fluid replacement with glucose-saline, on the responses to haemorrhage (30% of blood volume) in dogs, was investigated. Addition of metaraminol significantly increased the blood sugar and lactate responses to haemorrhage; the lactate-pyruvate (L/P) ratio, plasma phosphate and free fatty acid (FFA) levels were also significantly increased. A delayed hypotension was observed. Responses to haemorrhage during infusion of glucose-saline + insulin showed reduced blood sugar, blood lactate, pyruvate, and L/P ratios, plasma inorganic phosphate and FFA were diminished while, mean arterial blood pressure (MABP) changes were significantly higher than the glucose-saline group. Infusion of trasylol in glucose-saline did not significantly affect the blood sugar, lactate, and pyruvate responses and the L/P ratio. Plasma phosphate response was also like the control group. The responses of plasma FFA and of MABP in this group were significantly higher than the glucose-saline group.

The mechanisms of these effects and the significance of these results are discussed.

Introduction

Treatment of haemorrhage consists simply in restoration of the blood volume by giving type specific blood transfusion. However, greatest danger with the use of blood is the risk of transmitting viral hepatitis which is about 10% (Grindon, Holland & Schmidt, 1967). In most instances blood is not available and treatment must be started with an intravenous solution until it can be supplied.

For practical purposes premixed electrolyte solutions as glucose-saline, are regarded as a satisfactory intravenous fluid therapy in the clinical management of shock (Weil & Shubin, 1967).

In the present work it was thought of interest to investigate the potential values of adding three drugs namely: aramine (metaraminol), insulin and trasylol, unilaterally, in a fluid medium containing a crystalloid (glucose) and an electrolyte (0·9% NaCl); in an attempt to favourably influence the cardiovascular and metabolic responses to lesser grades of blood loss (30% of blood volume) which does not usually result in irreversible shock.

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Materials and methods

Twenty dogs of average weight 13 kg were randomly allocated into control and experimental groups. After an overnight fast, animals, except group 2, were anaesthetized with thiopentone sodium (nesdonal) 30–50 mg/kg i.v. initially, followed by smaller doses as required to maintain anaesthesia. Owing to the frequent occurrence of severe hypotension and sometimes apnoea during catecholamine infusion in dogs under thiopentone anaesthesia (Zaki, Hani-Ayobe & Rifaat, 1974), dogs receiving metaraminol (group 2) were anaesthetized with sodium pentobarbitone at a dose of 30 mg/kg by wt. i.v. Following the induction of anaesthesia mean arterial blood pressure was recorded from the femoral artery through a catheter connected to a mercury manometer and the animals were bled through a cannula

inserted into the opposite femoral artery. Infusions were given through a catheter inserted into one of the femoral veins.

Experimental protocol

Dogs were divided into the following groups.

Group 1. (5 *dogs*), *Dextrose-saline alone*

Animals in this group were given a continuous i.v. infusion of 5% dextrose in normal saline at a rate of 100 ml per h. Allowing a 15 min period for equilibrium to be established, base-line blood pressure tracings were monitored. A control (0 min) pre-haemorrhage blood sample was taken and 1 min later dogs were subjected to sudden removal of whole blood 30% of blood volume i.e. 27 ml/kg b. wt. (Ehrlich, Kramer & Watkins, 1969) collected over a period of 15 min. Blood specimens were drawn at 15, 30, 45, 60, 75, 105 and 135 min from the start of bleeding. This group served as a control.

Group 2 (5 *dogs*), *dextrose-saline + aramine*

In this group in addition to glucose-saline

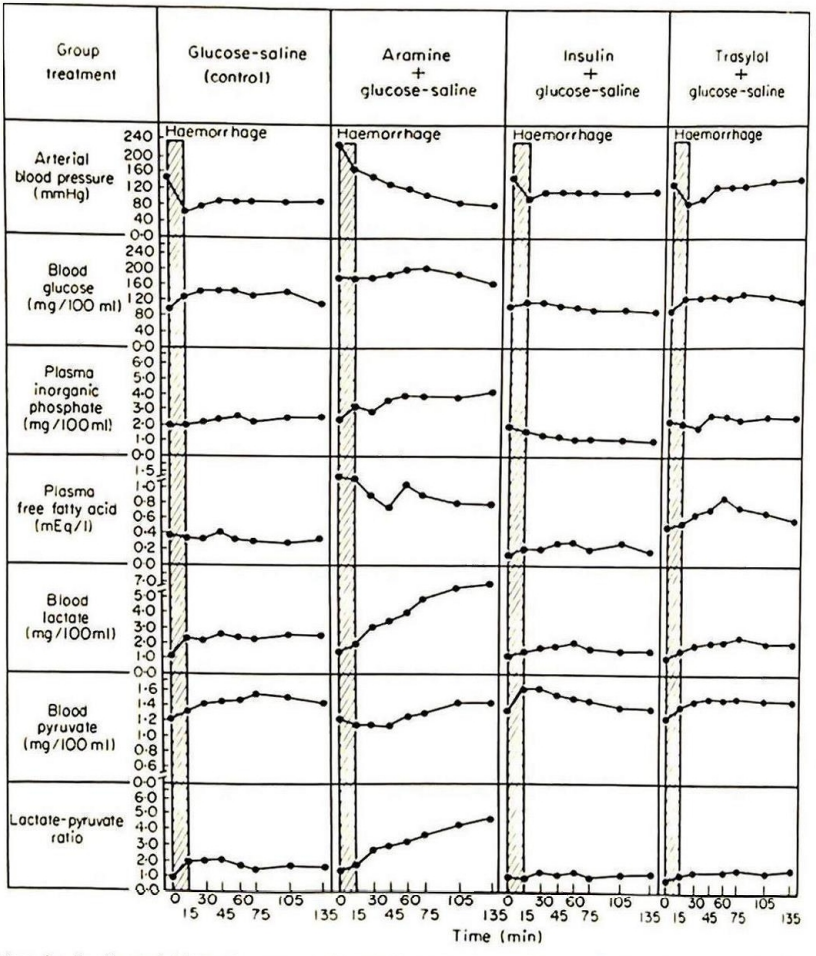

FIG. 1. Mean levels of arterial blood pressure, blood glucose, plasma inorganic phosphate, etc., following haemorrhage (30% of blood volume) in dogs receiving various regimens of replacement therapy. Values are the means statistically significant differences are described in the text.

aramine (metaraminol) 10 ml of 1% solution per 500 ml, was administered at the same rate of 100 ml/h as in group I. After taking baseline blood samples, animals were bled 30% of blood volume over a 15 min interval. Blood samples were collected exactly as described in the above group.

Group 3 (5 *dogs*), *dextrose-saline + insulin*

The infusion fluid in this group contained in addition to glucose-saline, soluble insulin (Leo) 50 u/500 ml. The procedures of bleeding and blood sampling were carried out as described in the above groups.

Group 4 (5 *dogs*), *dextrose-saline + trasylol*

The infusion fluid in this group contained trasylol (Bayer), a kallikrein inhibitor obtained from bovine parotid gland, in a concentration of 50,000 KIU per 500 ml dextrose-saline. Hypovolaemia was produced in the same manner as in the other groups. Blood specimens were also taken at 0, 15, 30, 45, 60, 75, 105 and 135 min from the onset of haemorrhage.

Analytical procedures

Samples of blood were drawn into chilled tubes containing heparin. Half ml amounts were taken for glucose determination by the Nelson-Somogyi (1944) method. One ml portions were immediately deproteinized for estimation of blood lactate and pyruvate according to Barker & Summerson (1941) and Friedemann & Haugen (1943) respectively.

Samples were centrifuged within 30 min from collection and plasma separated. Plasma inorganic phosphate was estimated by the Kuttner & Lichtenstein (1930) method; and plasma free fatty acids (FFA) was determined as described by Itaya & Ui (1965).

Statistical Analysis

The intraindividual changes in the parameters tested, before and after haemorrhage were compared by Student's '*t*' test for paired data. For comparisons between the different groups, Student's '*t*' test for non-paired data was used.

Results

The cumulative data obtained in these groups of dogs are graphically illustrated in Fig. 1.

Effects of dextrose-saline infusion (*Group* 1)

Removal of 30% of blood volume in dogs under continuous infusion of dextrose-saline, resulted in a sudden drop of mean arterial blood pressure (MABP) from a baseline level of 145–60 mmHg in the first 15 min. From the end of bleeding and for 2 h. thereafter, the MABP showed a gradual rise, though still significantly below the initial levels.

Metabolic response

Hyperglycaemia, lactacidaemia, pyruvic acidaemia and increased ratios of lactate-pyruvate were observed. The plasma FFA response was not altered significantly throughout the observation period. The rise of plasma inorganic phosphate following haemorrhage was statistically insignificant through the period 15–135 min, except at 105 min when the response was significant ($P < 0{\cdot}02$).

Effects of aramine (*metaraminol*) *plus dextrose-saline* (*Group* 2)

Hypovolaemia in this group produced also a significant drop of MABP, that was progressive until the end of the observation period. By comparison with group 1, it can be appreciated that despite the significantly higher ($P < 0{\cdot}001$) initial MABP in this group, the values gradually approached those of group 1 and became insignificant at 45–135 min following bleeding. Dogs in this group were much more stressed by this degree of hypovolaemia than the other groups.

Metabolic response

The glycaemic response to blood loss in this group as well as the baseline blood sugar levels were much higher than group 1 dogs. A significant hyperphosphataemia was displayed by these animals. The plasma FFA levels were significantly higher than group 1 at all times 0–135 min. In response to haemorrhage a gradual and progressive decrement, with a nadir at 45 min, was seen at times 15–135 minutes. This effect reached statistical significance at 45–105 min following bleeding. The blood lactate following infusion of aramine was not altered significantly from the baseline value in control dogs (group 1). After induction of bleeding the blood lactate rose progressively, to reach significant levels

at 30 and 45 min and as well all through the period 75–135 min. This response was higher than that of group 1.

The baseline pyruvate levels did not differ from group 1, but the response within the group was biphasic consisting of an initial reduction in the first 45 min. This was followed by a gradual rise significant at 75–135 min. In comparison with group 1, the lower pyruvate response was statistically significant at 30–75 min. The lactate-pyruvate (L/P) ratios were significantly increased within the group throughout the posthaemorrhage period.

Effects of insulin plus dextrose-saline (group 3)

The results of blood pressure in this group, indicate that the drop of MABP at the end of haemorrhage, was less than group 1 (initial levels 145 mmHg before and 94 mmHg at the end of bleeding). Also the MABP was higher than the corresponding values in group 1, throughout the period 30–135 min. This effect reached significant level at times 105 and 135 min.

Metabolic response

Induction of haemorrhage in this group was followed by a normoglycaemic response, as there was no statistically significant differences between the baseline blood glucose values and the levels following bleeding. This response was accompanied by a pattern of hypophosphataemia, characteristic of the insulin effect. Compared to group 1 this effect was significant all through the time interval 45–135 min.

Also due to the action of insulin, baseline FFA levels were significantly reduced below group 1 ($P < 0.005$). Though the response of FFA to haemorrhage did not show any significant difference from the glucose-saline group (group 1), yet on the basis of analysis within the group, the FFA levels progressively increased above baseline following bleeding. This lipolytic effect was statistically significant at 60–135 min. The blood lactate and pyruvate responses showed a mild rise, occasionally significantly different from baseline values. The L/P ratios were insignificantly raised, except at 60 min ($P < 0.05$) and 135 min ($P < 0.005$). The responses in these three parameters were in general not significantly different from group 1.

Effects of trasylol plus dextrose-saline (group 4)

Hypovolaemia in dogs under continuous infusion of the kallikrein inhibitor-trasylol-produced a significant drop of MABP from an initial level of 135 mmHg to 80 mmHg by the end of bleeding. However, within 1 h, from the onset of haemorrhage, the MABP returned to near baseline values and became insignificantly different from it, at all the periods 60–135 min. MABP values were significantiy increased above those in the control group all through the time interval 75–135 min.

Metabolic response

A mild but significant hyperglycaemia was observed in this group 15–105 min and returned to insignificant levels at the end of the observation period. These blood-sugar changes were not statistically different from group 1 dogs. The plasma phosphate, likewise, differed insignificantly from control group, but within the group a significant hypophosphataemia ($P < 0.025$) was seen at 15 and 30 min from the start of bleeding.

Mean plasma FFA levels in this group rose progressively following induction of hypovolaemia with a peak value at 60 min and then a gradual decline to values approaching baseline levels. This response was significant at 60–105 min and by comparison with the control group the rise of plasma FFA was statistically significant at 15–105 min. This FFA response as seen in Fig. 1, resembles that observed in dogs receiving insulin. The blood lactate, pyruvate and L/P ratios, behaved exactly as the control group.

Discussion

The present data have shown that infusion of metaraminol in glucose-saline (MGS regimen), maintained a satisfactory level of arterial blood pressure, although hypotension developed later. By using this regimen the metabolic responses to haemorrhage were aggravated. These results extend the observations that sympathomimetic drugs quicken the onset of irreversibility (Schumer & Durani, 1963) despite their beneficial effects on the myocardium (Goldenberg *et al.*, 1949 and Swan 1951).

Infusions of insulin in glucose-saline, (IGS regimen), on the other hand, produced a significant hemodynamic improvement as measured by the

blood pressure response, and improved the diabetogenic effects of haemorrhage. The beneficial effects of this regimen may be ascribed to the hypokalemic action of insulin thus, reducing the cardiotoxicity of excess K^+. Also insulin corrects the failure of sodium pump that was shown to accompany haemorrhage (Fuhrman, 1960). These findings corroborate those of Spigelman & Ozeran (1970) who showed that insulin and glucose increased survival time in dogs during irreversible haemorrhagic shock.

The results of studies on the potential value of adding the kallikrein inhibitor, trasylol, to glucose saline (TGS regimen), have demonstrated a significant rise of MABP compared to control group. Moreover, the intraindividual drop of MABP following haemorrhage was reversed. These haemodynamic responses were achieved without adverse effects on the metabolic responses.

There are several explanations for the benefits of trasylol observed in this study. First, it reduces the excessive proteinase activity shown in shock resulting from haemorrhage (Webster & Clark, 1959; Meyer, 1966; Rothschild & Castania, 1968). Secondly, by decreasing the release of the myocardial depressant factor (MDF), isolated from blood of animals in haemorrhagic shock (Lefer *et al.*, 1967); it protects the heart from the deleterious effects of this peptide. Thirdly, being an anticoagulant substance (Dubber *et al.*, 1968), it prevents the hypercoagulability which accompanies haemorrhagic shock (Lasch, 1969), and serves to maintain adequate tissue perfusion.

The plasma FFA responses to haemorrhage in the groups of dogs studied followed two patterns; increased plasma levels in animals receiving glucose-saline plus insulin and trasylol, and a significant reduction of plasma, FFA in dogs receiving glucose-saline-metaraminol. These responses are of interest and their explanations are only speculative.

Growth hormone is secreted in response to haemorrhage (Mayer & Knobil, 1967) and glucose is known to suppress its secretion following a variety of stimuli (Catt, 1970). On the other hand, alpha adrenergic agents and inhibition of insulin secretion enhance growth hormone release (Werrbach *et al.*, 1967). In light of these facts we may propose that, the reduction of plasma FFA following haemorrhage during aramine infusion, may be due to the enhanced growth hormone action which exerts an antilipolytic effect in dogs (Rathgeb *et al.*, 1970), while the lipolytic response to haemorrhage in dogs given IGS and TGS, may be ascribed to inhibition of growth hormone response by glucose.

Conclusions

From the present findings we may conclude that metaraminol is deleterious in this degree of blood loss, while insulin and trasylol are beneficial and both drugs are recommended for clinical trial.

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