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PAPAVERINE-INDUCED METABOLIC ALTERATIONS IN PERFUSED CANINE AND PORCINE LIVER

MARY J. RUWART, DONALD L. KAMINSKI and JOHN W. HAHN
Department of Surgery, St. Louis University School of Medicine, St. Louis, MO 63104, U.S.A.

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Abstract—Papaverine was given to perfused porcine and canine livers to assess the effect of this antispasmodic on hepatic viability. Papaverine decreased oxygen consumption, portal venous resistance (PVR), and perfusate pyruvate while increasing perfusate lactate. After approximately 10 min, these parameters, except for perfusate lactate, began to return to their pre-papaverine values. The reduction of oxygen consumption accompanied by the output of lactate indicated a shift to anaerobic metabolism resulting from papaverine administration. These phenomena occurred in both species even though the reduction in PVR was much less pronounced in the porcine liver. The decrease in perfusate pyruvate was found to depend on pre-existing levels of pyruvate in the perfusate; the equations governing this behavior were similar in the canine and porcine livers. There was no correlation between change in lactate output and initial values of lactate; however, the change in perfusate lactate could be correlated to the change in perfusate pyruvate. Thus, the changes in pyruvate and lactate occurring upon papaverine administration are related to initial perfusate pyruvate, the levels of which may reflect the redox state of the hepatocytes. Papaverine did not improve the perfusion characteristics of porcine livers other than to slightly decrease PVR. Irreversible outflow block could be prevented or delayed by papaverine in canine preparations, however, when this drug was given at the onset of PVR rise. Thus, papaverine may be effective in perfusion and preservation of canine livers and other organs which undergo phenomena similar to outflow block due to venous sphincters.

The smooth muscle dilator, papaverine, directly affects various metabolic processes as well as acting as an antispasmodic. In polymorphonuclear lymphocytes, papaverine inhibited phagocytosis and respiration, while stimulating glycolysis and subsequent lactate production [1]. Papaverine almost completely abolished respiration and phosphate uptake of rat liver mitochondria oxidizing glutamate but not succinate [2]. Thus, inhibition of electron transport by papaverine is implied. Respiration and ATP content were lowered in C-6 astrocytoma cells treated with papaverine, whereas glycogenolytic activity of papaverine paralleled the well-documented [3–7] phosphodiesterase inhibition by this drug in rat diaphragm [4]. The increase in cyclic AMP has been proposed as a mediator of the muscle relaxant properties of this compound [8,9]. However, papaverine has also been shown to stabilize liver lysosomes *in vitro* which might be attributable to a direct interaction between the membrane and the antispasmodic [10].

Assuming that these various metabolic alterations in addition to vascular dilation would benefit the perfused organ *in vitro*, canine and porcine liver perfusions were performed. The basic difference in these two species is the presence of hepatic vein sphincters in the canine preparation which makes this species particularly susceptible to outflow block [11]. Thus, comparison of these species would enable us to assess whether the effects of papaverine in the liver are due to dilation or metabolic alteration.

MATERIALS AND METHODS

Mongrel dogs (15–19 kg) and young pigs (15–32 kg) fasted for 18 hr were used as liver donors. The hepatectomy and whole blood perfusion were performed as previously described [12]. The isolated perfused organs received oxygen-saturated blood via the portal vein and hepatic artery. The bile duct was cannulated and the cystic duct tied. Both blood vessels were supplied from the same "arterial" reservoir. The organ was normothermically maintained for 2–4 hr.

Papaverine (Eli Lilly) was injected in 150-mg doses into the "arterial" reservoir where it was mixed with the whole blood perfusate and delivered in identical concentrations to the portal vein and hepatic artery. Papaverine was given to livers after the preparation had stabilized, or, where specified, at the time of portal venous resistance (PVR) increase in canine livers. Since flows were maintained constant, after stabilization, changes in PVR reflected variations in perfusion pressure only.

Throughout the perfusion, blood was collected from the inflow and outflow sides of the liver for discrete and continuous on-line analyses. Arterial and venous oxygen, lactate, and pyruvate concentrations were continuously monitored on line [13] and the data relayed to a magnetic tape for computerized calculation of output. Glucose was measured discretely on arterial and venous plasma with an automated neocuprine method [14].

The concentration of sodium taurocholate in the arterial reservoir was assayed continously by a modification of the hydroxysteroid dehydrogenase method [15] previously described [12]. The rate of sodium taurocholate infusion was manually adjusted to maintain a constant flow of 20 μmoles/min to the liver.

Bile was collected in conical graduated centrifuge tubes at 10-min intervals. Bicarbonate concentrations were measured by titration [16]. Bile salt and chloride

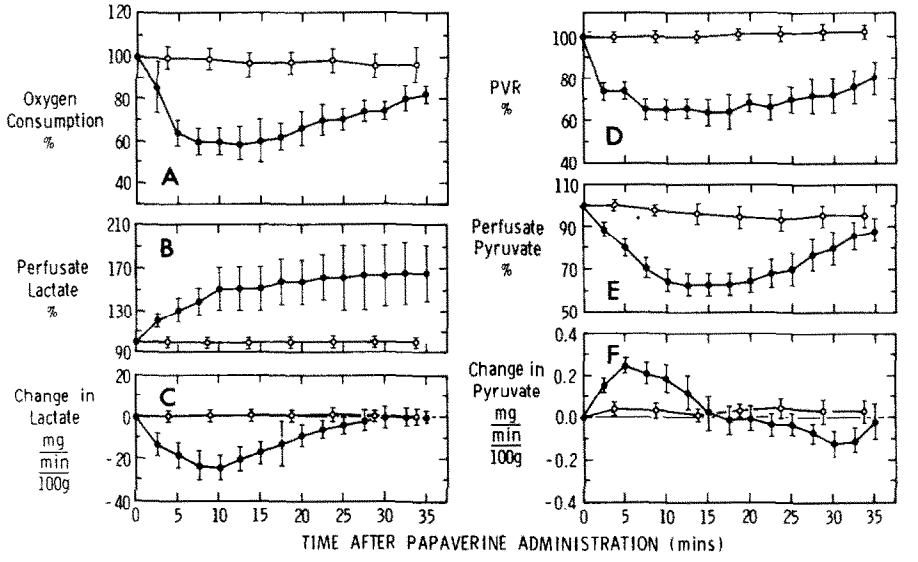

Fig. 1. Metabolic parameters in seven control canine livers (○) and seven livers given 150 mg papaverine (●) at time = 0 min. (A). Per cent change in oxygen consumption from initial values. (B). Per cent change in perfusate lactate from initial values. (C). Consumption (positive values) or output (negative values) of lactate expressed as mg/min/100 g of liver. (D). Per cent change in PVR from initial values. (E). Per cent change in perfusate pyruvate from initial values. (F). Consumption (positive values) or output (negative values) of pyruvate expressed as mg/min/100 g of liver.

concentrations were determined autoanalytically [16, 17]. Sodium and potassium were measured on a Jarrell–Ash atomic absorption photometer.

All calculations were performed on an Olivetti 652 computer interfaced to transfer the data from magnetic tape to disc storage.

RESULTS

Changes in metabolic and functional parameters of stable canine and porcine liver perfusions with and without papaverine administration are illustrated in Figs. 1 and 2. Oxygen consumption (Fig. 1A and 2A) was decreased to approximately 60 per cent of its pre-papaverine value, an effect which was reversed 30 min after papaverine administration. Perfusate lactate (Fig. 1B and 2B) was increased to 160 per cent (canine) and 190 per cent (porcine) of its initial value, stabilizing at that level approximately 15 min after administration. Lactate output by the liver (Fig. 1C and 2C) followed a pattern similar to the decrease in

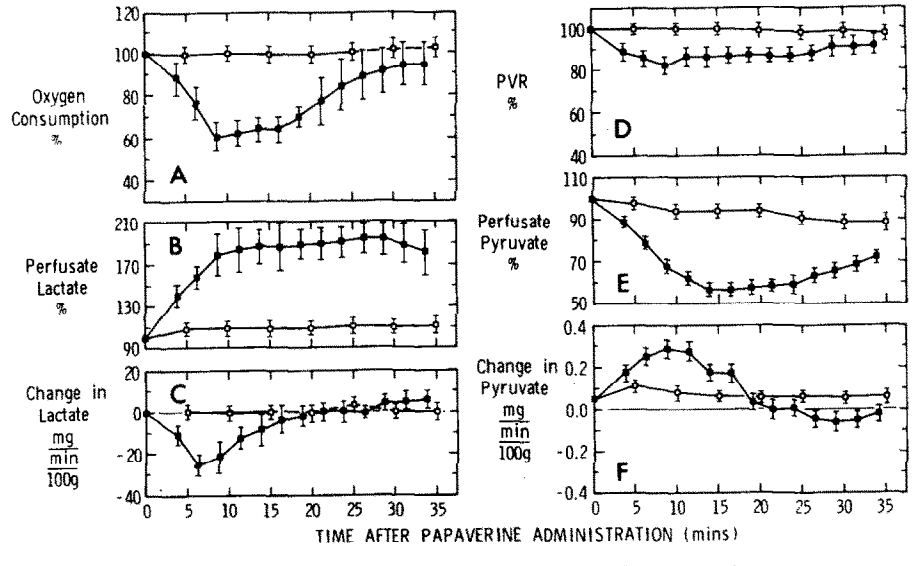

Fig. 2. Metabolic parameters in four control porcine livers (□) and four livers given 150 mg papaverine (■) at time = 0 min. (A). Per cent change in oxygen consumption from initial values. (B). Per cent change in perfusate lactate from initial values. (C). Consumption (positive values) or output (negative values) of lactate expressed as mg/min/100 g of liver. (D). Per cent change in PVR from initial values. (E). Per cent change in perfusate pyruvate from initial values. (F). Consumption (positive values) or output (negative values of pyruvate expressed as mg/min/100 g of liver.

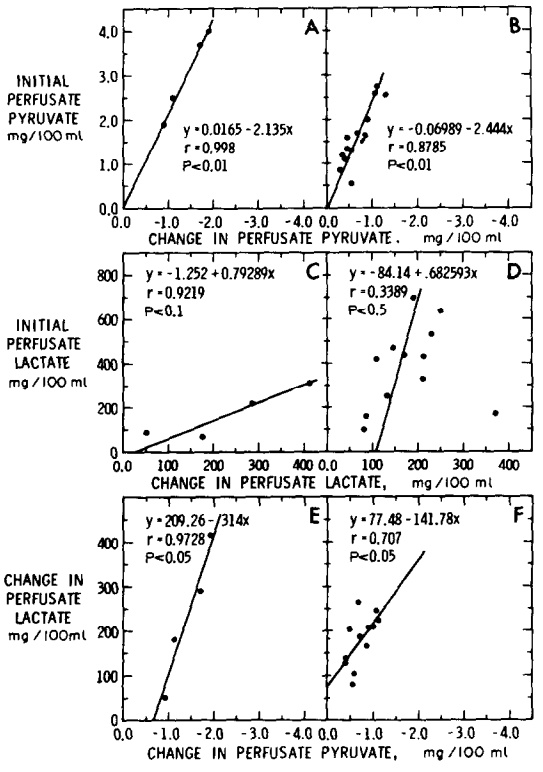

Fig. 3. Correlations between various perfusate levels and changes in perfusate levels of lactate and pyruvate at maximal papaverine effect. Panels A (porcine) and B (canine): relationship between initial perfusate pyruvate prior to papaverine administration and maximal decrease in perfusate pyruvate produced by papaverine administration. Panels C (porcine) and D (canine): relationship between initial perfusate lactate prior to papaverine administration and maximal increase in perfusate lactate produced by papaverine administration. Panels E (porcine) and F (canine): relationship between the maximal increase in perfusate lactate produced by papaverine administration and the maximal decrease in perfusate pyruvate by papaverine.

oxygen consumption (Fig. 1A and 2A). The porcine preparations showed less PVR fluctuation (Fig. 2D) than the canine livers (Fig. 1D). Perfusate pyruvate (Fig. 1E and 2E) dropped as pyruvate was initially consumed (Fig. 1F and 2F) and rose as the process was reversed. Thus, perfusate pyruvate, unlike perfusate lactate, returned to its pre-papaverine value as the effect of the drug lessened.

Thus, the initial effect of papaverine was to lower the PVR, oxygen consumption, and perfusate pyruvate and to increase perfusate lactate. After approximately 10 min, the effect of the papaverine began to diminish and the oxygen consumption, PVR, and perfusate pyruvate began to return to normal values. The new level of perfusate lactate did not diminish.

To determine whether the alterations of pyruvate and lactate in the perfusate were related or simply concurrent phenomena, the changes in the perfusate quantities were compared with each other and with initial perfusate concentrations (Fig. 3). Minimal values of perfusate pyruvate correlated well with the levels of pyruvate present before papaverine administration (Fig. 3A and 3B), suggesting that the pyruvate uptake seen with papaverine administration was a first-order process. The slope of the two lines governing this behavior was not significantly different ($P < 0.4$) between species.

Changes in perfusate lactate could not be significantly correlated with initial concentrations of this metabolite (Fig. 3C and 3D) in porcine or canine preparations. Changes in perfusate lactate could, however, be correlated with changes in pyruvate concentration (Fig. 3E and 3F); however, the equations governing this behavior were significantly different in the porcine and canine preparations ($P < 0.05$).

The metabolic and/or antispasmodic effects produced by papaverine appeared to benefit the perfused canine liver. This organ often exhibits rapid outflow block due to the presence of hepatic venous sphincters [18]. This syndrome is characterized by rapidly increasing PVR, large weight gain, darkening, decreased oxygen consumption, increased pyruvate output, and decreased bile flow. Within minutes of the onset of outflow block, the deteriorating condition is irreversible. Failing organs receiving papaverine, however, often ceased gaining weight, regained normal coloration, and often increased bile flow upon papaverine administration (Table 1). Even after the metabolic effects of the drug dissipated, the liver would retain normal perfusion characteristics for 1–2 hr. Porcine preparations do not usually exhibit rapid outflow blocks; thus, improvement in these parameters, except for PVR, was not observed.

No changes in perfusate potassium or glucose were detected upon papaverine administration. Bile flow was increased only in failing canine preparations, and no changes in bicarbonate, sodium, potassium, chloride, or bile salt concentrations were detected in bile secreted after papaverine administration.

DISCUSSION

Papaverine, an effective antispasmodic in isolated perfused canine liver, altered oxidative metabolism appreciably. Porcine preparations, although less sensitive to the antispasmodic properties of this drug, nevertheless exhibited similar metabolic alterations. These effects were probably not due to papaverine degradation, although the liver is the major site of its metabolism [19]. The degradation of papaverine requires oxygen, and, thus, it is evident that the net effect of papaverine upon oxygen consumption is not due to its detoxification. Decrease in oxygen consumption upon papaverine administration has been observed in other systems [2, 20] and has been linked to depression of the electron transport chain function [2]. High doses of papaverine can almost completely abolish respiration [2]. The increase in the lactate:pyruvate ratio in the perfusate seen upon papaverine administration is a further indication that the liver is shifting to a more anaerobic metabolism.

Inhibition of the electron transport chain might result in a transient buildup of NADH, while increased glycogenolysis may require additional NAD. This may be the reason for the pyruvate uptake, which can readily be converted to lactate with regeneration of NAD. NAD is not necessary, furthermore, for the enzymatic degradation of papaverine and is a poor substitute for the required cofactor

Table 1. Influence of papaverine on viability parameters of perfused canine livers after onset of outflow block*

Viability parameter	Control (10)	Papaverine (10)
Portal venous resistance—increasing (PVR)	10	2
—decreasing or stable	0	8
Bile flow—decreasing	10	3
—increasing or stable	0	7
Color—darkening	10	2
—reddening or stable	0	8
Weight gain—increasing	10	2
—decreasing or stable	0	8
Average time between onset and increase in portal venous pressure to 30 cm blood	15 ± 5 min	70 ± 20 min

* Parameters were measured 20 min after onset of outflow block (i.e. the first detectable rise in PVR after transfer of the liver to the perfusion apparatus). Papaverine was given in the designated group 5 min after onset. Controls received no treatment. The numbers in parentheses indicate the number of experiments.

NADP [19]. Thus, the NAD, if being generated, is probably not directly liked to the degradation of the administered drug, but rather to the new metabolic demands of the treated organ.

It would appear that limiting electron transport would result in an energy shortage and subsequent hepatic failure. However, the canine preparations visibly improved and remained in good condition after the effect of papaverine had diminished, whereas experience dictates that the same preparations not receiving the drug would certainly fail (see Table 1).

Two effects which might have offset the inhibition of the electron transport system were observed. The decrease in PVR may have resulted in better perfusion, even though flows were kept constant. The second phenomenon which may be beneficial to the perfused liver is the increase in anaerobic glycolysis suggested by the increased lactate output by the liver. Since lactate output was much greater than pyruvate uptake and there were no changes in perfusate glucose, it is assumed that the lactate must have come from pre-existing glucose or glycogen stores in the liver. This phenomenon might compensate for the decrease in energy available from electron transport activity. Although the concentration of glucose in media of polymorphonuclear leukocytes affects lactate production by papaverine [1], no correlation could be made in perfused liver between perfusate glucose and changes in perfusate lactate.

The correlation between the initial perfusate pyruvate and the change in perfusate pyruvate indicates that approximately 50 per cent of the available metabolite is consumed. The similarity of this consumption in both porcine and canine livers suggests that the process is common to both species. Although lactate output was related to pyruvate uptake in both species, the strong correlation between species was absent. More than twice as much lactate was released from the porcine liver compared to the canine liver at the same level of pyruvate uptake. Comparison between quantitative metabolic parameters of canine and porcine liver perfusions must be made with some caution, however. Due to the experimental design, dosage of papaverine could not be corrected for organ weight before drug administration. The porcine livers averaged 20 per cent heavier than the canine livers and thus a dosage phenomenon cannot be ruled out.

The action of papaverine is complex. However, these experiments demonstrate that the metabolic changes produced by this drug are independent of extensive vasodilation. The preservation of the canine liver, but not the porcine liver, can be extended by administration of papaverine during early outflow block, suggesting that the dilatory properties of this drug are responsible for the beneficial effects on canine liver.

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