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THE PRESENCE OF β-ADRENERGIC RECEPTORS IN THE HEPATIC VASCULATURE

BY

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The liver is ideally equipped to play a prominent part in systemic adjustments in both physiological and pathological conditions. By accommodating one-quarter of the total cardiac output per minute (Bender & Horvath, 1963, 1965), and by holding 25–30% of the total blood volume, the liver is the principal determinant of the venous return to the heart (Coleridge & Hemingway, 1958 ; Knisely, Harding & Debacker, 1957).

The innervation of the liver vasculature seems to be exclusively derived from the sympathetic nervous system. The first proof for the existence of a vasomotor mechanism within the liver was that of François-Frank & Hallion in 1896. They found that stimulation of the sympathetic supply caused a rise of hepatic and portal pressures and shrinkage of the liver. No other drug or group of drugs has been so extensively investigated in the hepatic circulation as the catecholamines and related drugs. Studies have been performed on isolated preparations as well as in intact animals and man with differing results. In the isolated perfused liver, both adrenaline and noradrenaline cause reduction in flow (Bauer, Dale, Poulson & Richards, 1932 ; Chakravarti & Tripod, 1940 ; Daniel & Prichard, 1951 ; Andrews, Hecker, Maegraith & Ritchie, 1955 ; Andrews Hecker & Maegraith, 1956). This response is blocked by ergotoxine (Bauer *et al.*, 1932), azapetine, and phenoxybenzamine (Green, Hall, Sexton & Deal, 1959). In intact animals and in man, however, adrenaline can apparently lead to an increase of hepatic blood flow, possibly secondary to a rise in blood pressure (Clark, 1928 ; Bearn, Billing & Sherlock, 1951 ; Bender & Horvath, 1963, 1965). In the liver, only α-adrenergic receptors were detected in hepatic arterioles and portal venules (Green *et al.*, 1959).

The present investigation was undertaken to demonstrate the presence of β-adrenergic receptors in the hepatic arterial and portal venous beds of the isolated perfused canine liver.

METHODS

Twenty male dogs weighing 8–20 kg were used. Anaesthesia was induced and maintained by ether for the time taken to isolate the liver. Through a midline abdominal incision the hepato-duodenal artery was exposed and cannulated with a fine cannula. All other arterial branches were ligated so that all perfusion into the hepato-duodenal artery would pass into the liver. The main tributaries of the portal vein were ligated and a cannula was inserted in the portal vein immediately before its entry into the liver. An 8% solution of chlorazol fast pink in a dose of 1 ml./kg was administered through the cannulated femoral vein to prevent blood clotting.

Perfusion was performed with freshly prepared Tyrode solution (gassed with 95% oxygen and 5% carbon dioxide) through the portal vein and the hepatic artery. Physiological perfusion pressures (in the hepatic artery 130 mm Hg, and in the portal vein 10 mm Hg), temperature (37.5° ±0.5° C) and pH (7.35) were maintained. The thorax was then opened. The outflow cannula was inserted in the hepatic vein. The abdominal inferior vena cava was ligated above the level of the renal veins. By this means, the liver was completely isolated from the general circulation but was left *in situ*. The dog was bled to death while the liver was perfused with Tyrode solution. The perfusion apparatus and maintenance of the isolated perfused canine liver have previously been reported in detail (Geumei, 1964; Mahfouz & Geumei, 1965). The liver was perfused simultaneously through both the hepatic artery and the portal vein and the perfusate was collected from the hepatic vein. This is an open-circuit perfusion technique; no outflow block was observed. Each experiment had its own control. Hepatic arterial and portal venous flows per minute were measured before the use of the drug to be tested. The liver was then perfused through the hepatic artery only and the perfusate was collected from both hepatic and portal veins—that is, the portal vein acted as an outflow tract. The ratio of the portal vein outflow to the total hepatic outflow (PVOR) was calculated.

This isolated perfused liver preparation has been repeatedly shown to preserve both structure and function during prolonged periods of perfusion (Geumei, 1964) and so it is a suitable method for the study of the effects of drugs on hepatic circulation (Mahfouz & Geumei, 1965, 1967; Geumei, Mahfouz & Issa, unpublished). Most of the experiments were terminated after about 3 hr.

If constant perfusion pressures are maintained in the hepatic artery (130 mm Hg) and in the portal vein (10 mm Hg) (Mahfouz, Shafei & Geumei, 1960), an increase in flow can be safely considered to indicate the occurrence of vasodilatation and a decrease in flow to denote vasoconstriction.

The effect of 2 μg/ml. of the β-adrenergic stimulating agent nylidrin HCl (phenyl secondary butyl nor-hydroxyephedrine) on hepatic arterial and portal venous vascular beds was studied in fifteen preparations. In five other isolated livers, the effect on both hepatic arterial and portal venous beds of the β-adrenergic stimulating agent was observed before and after the administration of a β-adrenergic blocking agent, propranolol.

RESULTS

Four groups, each of five preparations of dog liver, were used.

Group 1

Perfusion with nylidrin solution 2 μg/ml. through the hepatic artery, and with normal Tyrode solution through the portal vein, increased hepatic arerial flow by 58% ±4.2% (standard error of the mean) of the control value. The portal venous flow diminished by 51% (S.E. 4.4) of the control value (Table 1). The ratio of the portal vein outflow to total hepatic outflow diminished from a normal value of 60% to 30% (Table 2).

TABLE 1

EFFECT OF PERFUSION IN THE HEPATIC ARTERY OF THE β-RECEPTOR STIMULANT NYLIDRIN HCl (2 μg/ml.) WHILE THE PORTAL VEIN IS PERFUSED WITH TYRODE SOLUTION

Standard error of the mean (S.E.M.).

	Hepatic arterial flow (ml./min)				Portal venous flow (ml./min)			
Exp. No.	Control	After Nylidrin	Difference from control	% change	Control	After Nylidrin	Difference from control	% change
1	100	170	+70	+70	260	140	−120	−46
2	80	120	+40	+50	240	120	−120	−50
3	100	150	+50	+50	300	100	−200	−67
4	90	150	+60	+67	270	130	−140	−52
5	80	125	+45	+56	250	150	−100	−40
Mean				+58				−51
S.E.M.				4·2				4·4
Significance				$P<0·01$				$P<0·01$

TABLE 2

EFFECT OF NYLIDRIN HCl (2 μg/ml.) PERFUSED THROUGH THE HEPATIC ARTERY AND THE PERFUSATE COLLECTED FROM BOTH HEPATIC AND PORTAL VEINS

Standard error of the mean (S.E.M.).

	Control				After nylidrin perfusion			
		Total hepatic outflow				Total hepatic outflow		
Exp. No.	Hepatic arterial inflow (ml./min)	Hepatic venous outflow (ml./min)	Portal venous outflow (ml./min)	PVOR (%)	Hepatic arterial inflow (ml./min)	Hepatic venous outflow (ml./min)	Portal venous outflow (ml./min)	PVOR (%)
1	100	40	60	60	170	100	70	41
2	80	30	50	62·5	120	90	30	25
3	100	40	60	60	150	100	50	33
4	90	40	50	55	150	115	35	23
5	80	30	50	62·5	125	90	35	28
Mean				60				30
S.E.M.				1·3				3·2

Group 2

Perfusion of the liver with nylidrin solution 2 μg/ml. through the portal vein while the hepatic artery was perfused with normal Tyrode solution did not change the hepatic arterial flow. The portal venous flow decreased by 86% (S.E. 2.0) of the normal mean control value (Table 3).

TABLE 3

EFFECT OF THE β-RECEPTOR STIMULANT NYLIDRIN HCl (2 μg/ml.) PERFUSED IN THE PORTAL VEIN, WHILE THE HEPATIC ARTERY IS PERFUSED WITH TYRODE SOLUTION

Standard error of the mean (S.E.M.).

	Hepatic arterial flow (ml./min)				Portal venous flow (ml./min)			
Exp. No.	Control	After nylidrin	Difference from control	% change	Control	After nylidrin	Difference from control	% change
1	80	80	0	0	400	80	−320	−80
2	140	140	0	0	450	45	−405	−90
3	100	100	0	0	300	50	−250	−83
4	80	80	0	0	220	20	−200	−91
5	90	90	0	0	270	40	−230	−85
Mean								−86
S.E.M.								2·0
Significance								$P<0·01$

Group 3

Perfusion of the liver through both the hepatic artery and portal vein with the β-adrenergic stimulating agent increased the hepatic arterial flow by 57% (S.E. 4.1) of the mean control value, and diminished the portal venous flow by 88% (S.E. 2.0) of the normal mean control value (Table 4). The portal vein outflow ratio decreased from a control value of 55% to 25% (Table 5).

Group 4

Perfusion of the liver with nylidrin solution 1 μg/ml. through the hepatic artery, and with Tyrode solution in the portal vein, increased the hepatic arterial flow by 25%

TABLE 4

EFFECT OF THE β-RECEPTOR STIMULANT NYLIDRIN HCl (2 μg/ml.) PERFUSED IN BOTH HEPATIC ARTERY AND PORTAL VEIN

Standard error of the mean (S.E.M.).

	Hepatic arterial flow (ml./min)				Portal venous flow (ml./min)			
Exp. No.	Control	After nylidrin	Difference from control	% change	Control	After nylidrin	Difference from control	% change
1	75	125	+50	+67	220	20	−200	−91
2	60	90	+30	+50	170	10	−160	−94
3	60	90	+30	+50	270	40	−230	−85
4	80	135	+55	+68	260	40	−220	−86
5	100	150	+50	+50	300	50	−250	−83
Mean				+57				−88
S.E.M.				4·1				2·0
Significance				$P<0·01$				$P<0·01$

TABLE 5

EFFECT OF NYLIDRIN HCl (2 μg/ml.) PERFUSED THROUGH THE HEPATIC ARTERY AND THE PERFUSATE COLLECTED FROM BOTH HEPATIC AND PORTAL VEINS

Standard error of the mean (S.E.M.).

	Control				After nylidrin perfusion			
		Total hepatic outflow				Total hepatic outflow		
Exp. No.	Hepatic arterial inflow (ml./min)	Hepatic venous outflow (ml./min)	Portal venous outflow (ml./min)	PVOR (%)	Hepatic arterial inflow (ml./min)	Hepatic venous outflow (ml./min)	Portal venous outflow (ml./min)	PVOR (%)
1	75	35	40	53	125	100	25	25
2	60	30	30	50	90	60	30	33
3	60	30	30	50	90	70	20	22
4	80	30	50	62	135	100	35	25
5	100	40	60	60	150	120	30	20
Mean				55				25
S.E.M.				2·5				2·2

(S.E. 1.1) of the control value, the portal venous flow decreased by 50% (S.E. 4.8), and the portal vein outflow ratio decreased from a control value of 50% to 25%. Perfusion of the liver through the hepatic artery with propranolol solution 1 μg/ml. abolished the effect of nylidrin on the hepatic artery. The hepatic arterial flow returned to the control value, while the portal venous flow diminished further. Re-perfusion of the liver with the β-adrenergic stimulating agent nylidrin after β-adrenergic blockade with propranolol did not change the hepatic arterial flow, and the portal venous flow continued to diminish.

DISCUSSION

The adrenergic nervous system plays an important part in the control of many organ systems which function below the level of consciousness. In 1948, Ahlquist proposed that two distinct adrenergic receptors, α and β, mediate the responses to sympathomimetic amines in various effector organ systems. This classification by Ahlquist has been supported by studies in which selective blockade of the α or β receptors was produced by different drugs. In the liver, only α receptors were detected in hepatic arterioles and portal

venules. No β-adrenergic dilator responses in either hepatic vascular beds were observed (Green *et al.*, 1959).

In the recent study of the pharmacodynamics of hepatic circulation in our laboratory (1958–1964), nylidrin HCl was found to be peculiar in that it was, at that time, the only one of the well-known vasodilator agents tested which increased the hepatic arterial flow. The mechanism of its action was unknown (Geumei, 1964). Later, Frohlich & Schnaper (1964) suggested that nylidrin exerted its local and myocardial actions by stimulation of the β-adrenergic receptors.

The results described in the present investigation demonstrate that perfusion of the liver with nylidrin solution through the hepatic artery and Tyrode solution in the portal vein increased hepatic arterial flow and decreased portal venous flow. The ratio of the portal vein outflow to the total hepatic flow was decreased. Briefly, the portal vein outflow ratio denotes the difference between the resistance to flow in the portal vein and that in the hepatic vein. If the portal vein outflow ratio is increased, as after the administration of histamine or pitressin (Mahfouz & Geumei, 1967), this shows that the hepatic vein is more constricted than the portal vein and the unidirectional arterio-portal shunt (Geumei, Mahfouz & Aboul-Enein, unpublished). After perfusion of the liver with nylidrin solution, the portal vein and the arterio-portal shunt were much more constricted than the hepatic vein. So the fluid passes through the least resistant (least constricted) vessel—the hepatic vein—resulting in diminution of the portal vein outflow ratio.

On the other hand, perfusion of the liver with the drug solution through the portal vein and normal Tyrode solution in the hepatic artery did not affect the hepatic arterial flow although the portal venous flow diminished. This confirms the presence of a unidirectional hepatic arterio-portal shunt in the normal canine liver (Geumei, Mahfouz & Aboul-Enein, unpublished).

Recently, the introduction of propranolol, a strong β-adrenergic receptor blocking agent, has resulted in extensive evaluation of the role of β-adrenergic receptors in the regulation of the circulation (Black, Crowther, Shanks, Smith & Dornhorst, 1964 ; Shanks, 1964).

Perfusion of the liver with the β-adrenergic blocking agent propranolol after perfusion with nylidrin abolished the hepatic arterial effect of nylidrin ; that is to say, the hepatic arterial flow returned to the normal control value, and the portal venous flow was further diminished. Re-perfusion of the liver with the β-adrenergic stimulating agent nylidrin did not affect the hepatic arterial flow, but the portal venous flow diminished. Kaiser, Ross & Braunwald (1964) found that β-adrenergic stimulation produced veno-constriction which was abolished after β-adrenergic blockade by pronethalol (nethalide). The β-adrenergic blocking agent propranolol was found to constrict the portal vein. It has a direct action on the portal vein other than blocking the β-adrenergic receptors (Geumei, Mahfouz & Issa, unpublished).

These experiments demonstrate the presence of β-adrenergic receptors in the hepatic artery.

SUMMARY

1. The effects of the β-adrenergic stimulating agent nylidrin on hepatic arterial and portal venous beds have been studied in twenty isolated perfused dog livers.

2. Perfusion of the liver with the β-adrenergic stimulant drug through the hepatic artery, and with normal Tyrode solution in the portal vein, decreased both portal venous flow and portal vein outflow ratio to the total hepatic outflow. The hepatic arterial flow was increased.

3. Perfusion of the liver with the β-adrenergic stimulant through the portal vein and with normal Tyrode solution through the hepatic artery did not affect hepatic arterial flow, although the portal venous flow was diminished.

4. The hepatic arterial action of nylidrin was completely abolished after perfusion of the liver with the β-adrenergic blocking agent propranolol.

5. The presence of β-adrenergic receptors in hepatic vasculature was demonstrated. Stimulation of the β-adrenergic receptors in the liver increased hepatic arterial flow, and decreased portal venous flow and portal vein outflow ratio.

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