FACTORS PRODUCING VARIOUS RESPONSES TO ACETYLCHOLINE IN DOG HEPATIC CIRCULATION

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Abstract—Effect of acetylcholine on hepatic circulation was investigated in 33 mongrel dogs. Mean arterial blood pressure, hepatic artery flow, portal vein pressure and portal vein flow were determined after laparotomy under nembutal anesthesia. In order to analyse the complicated responses in hepatic blood flows to 3 μg of acetylcholine hydrochloride, the peripheral resistance of each vascular system was observed in hypotensive dogs, in vascular obliteration dogs and in portal vein shunting dogs. Observation under a hypotensive condition produced by exsanguination revealed that hepatic artery resistance responded to decrease in hepatic artery pressure in different ways depending on whether the systemic blood pressure was above or below 90 mmHg. Experiments in which the hepatic artery or portal vein were obliterated disclosed that there was a certain regularity in the induction of resistance change in the non-injected one of the two hepatic vessels. The result of shunting the portal vein suggested that the complicated change above mentioned would be the expression of the overlapping two sequential responses. Since these results are difficult to explain merely by the direct response of vascular smooth muscle to acetylcholine, the possibility that the hypothetical communication between the hepatic artery and portal vein might be a cholinergic system was discussed.

It has long been observed that the hemodynamics of the liver, which is supplied by the two vascular systems, the hepatic artery and portal vein, are somewhat different from those of any other organ (1–7, 10–24). Many authors have discussed the presence or absence of a blood flow regulation mechanism specific to the liver and have estimated the related properties, yet did not reach any definite conclusion.

On the other hand, the physiologic functions of adrenergic and cholinergic receptors, which are considered to be distributed on the vascular perfusion beds, that is, their pharmacologic reactions to noradrenaline, isoproterenol and acetylcholine were also studied by many investigators (6, 7, 8, 15, 21–23). The diversity among the reports on the distribution and the function of cholinergic receptors in the liver has also attracted the attention of many workers. It has been confirmed that the administration of a rather large dose of acetylcholine often produced different responses. Wong (8) termed the responses as atypical and discussed the mode of manifestation of the atypical responses. The present author attempted to elucidate the diversity in physiologic roles of cholinergic receptors distributed on the hepatic vascular wall by exploring the mechanism of these various responses to acetylcholine.

MATERIALS AND METHODS

Thirty-three male and female mongrel dogs, weighing 13.0–36.0 kg, were fasted 24 hr prior to the experiment, and were then anesthetized with pentobarital sodium (30 mg/kg,
The stage of anesthesia was kept nearly constant by adding the approximate dose of the anesthetic throughout the experiment. Heparin sodium 250 μg/kg i.v. was given at first, after which half the dose was given at 30 min intervals. The procedure was a modification of the method described by Kawazoe (7). The modification applied in the present experiment differed from the original in the following points: The apparatus measuring blood flow was the electromagnetic flowmeter (MF-6A, Nihon Kohden Co.) and a probe of cannulating type; as to operative technic, the gastroduodenal artery was ligated immediately after branching from the proper hepatic arteries, and cannulation was made at a distal site of the ligation for the perfusion of the distal with left femoral artery blood. The common hepatic artery was ligated on the side of the abdominal aorta, and the hepatic side was perfused with right femoral artery blood.

The blood pressure in the right femoral artery and the perfusion pressure in the portal vein were determined by high pressure and low pressure transducer (RP-S, Nihon Kohden Co. and LP-3, Toyomeas), respectively. The scheme for the operation is given in Fig. 1. For determination of the hepatic vein pressure, a silicon tube (1.5 mm in caliber) was inserted into the femoral vein and pushed through the inferior vena cava up to the opening of the hepatic vein beneath the diaphragm.

A state of hypotension was allowed to occur by exsanguination of 100–150 ml of the venous blood at the rate of 20 ml/min.

Three dogs were used for the observation on the peripheral resistances in the two vascular systems when the hepatic artery (HA) or the portal vein was obliterated in their extracorporal shunts with the clamp for 1 min.

A Sigma motor pump was set at a shunt connecting portal vein (PV) with femoral vein in 3 dogs, and thus an abrupt fall in the portal vein blood flow (PVF) was produced without MABP changes, in order to determine changes in the peripheral resistances on the side of PV and HA. Cannulation in the shunt was made at a point on the intestinal side of the flowmeter probe insertion.

The peripheral resistances of HA, PV and the mesenteric artery (RHA, RPV and RMA, respectively) were computed from blood pressure and blood flow rates by the following formulas.

\[
RHA = K_i \frac{MABP \text{ (mmHg)} - \text{Hepatic vein pressure} \text{ (mmHg)}}{HAF \text{ (ml/min)}}
\]

\[
RPV = K_i \frac{PVP \text{ (mmHg)} - \text{Hepatic vein pressure} \text{ (mmHg)}}{PVF \text{ (ml/min)}}
\]

Fig. 1. Schematic diagram for perfusion circuits, MABP = mean arterial blood pressure; PVP = portal vein pressure; HVP = hepatic vein pressure; HAF = hepatic arterial flow; PVF = portal vein flow.
Acetylcholine hydrochloride was injected into HA through the extracorporal shunt tube or into PV at the site connecting the probe in a dose of 3 μg.

**RESULTS**

MABP determined in 33 untreated dogs was 47.5–115.0 mmHg with the mean and the standard deviation of 109.5±22.4 mmHg. Portal vein pressure (PVP) was 90.0–277.5 mmH₂O with 182.7±48.4 mmH₂O. Hepatic vein pressure (HVP) was 59.7±22.7 mmH₂O. HAF was 52.5–155.0 ml/min with 82.7±22.9 ml/min. PVF was 108.0–475.0 ml/min with 251.7±99.6 ml/min. The hepatic blood flow (HIF = HAF - PVF) was 178.4–560.0 ml/min with 336.4±103.6 ml/min. The relation of HIF to HAF or PVF was investigated in the dogs above described with the following results: There was no significant relation between HIF and HAF. However, a significant correlation (P<0.01) was observed between HIF and PVF, with the correlation coefficient (r) of 0.973. When x-axis denotes HIF and y-axis PVF, y = 0.941x - 64.63 is obtained. Thus PVF = 0 theoretically when HIF = 68.69. A significant correlation (P<0.05) was also observed with r = 0.543 between MABP and HIF. When x-axis denotes HAF and y-axis MABP, a regression formula y = 0.72x - 41.19 is obtained and becomes 0 when MABP is 41 mmHg.

Fig. 2 showed the results of observation on the relationship between HAF and RHA in the fourteen hypotensive dogs. MABP dropped after exsanguination by 45.0±21.3 mmHg on the average from the intinal level and HAF by 28.6±20.4 ml/min. At the same time, the hepatic artery (IHA) and portal vein (IPV) pressures also showed significant changes.

**Table 1**

| MABP (mmHg) | PVP (mmHg) | RMA = Ki | PVF (ml/min) |
|-------------|------------|----------|--------------|
| K1: Coefficient including the internal resistance of blood |

**Fig. 2.** Changes in RHA and HAF after exsanguination. □: Before exsanguination, ●: After exsanguination.

**Fig. 3.** Curves for MABP, PVP, HAF, PVF, RHA, RPV and RMA following the administration of Acetylcholine (ACh) (3 μg) through the hepatic artery (IHA) or the portal vein (IPV).
time, changes were elicited in RHA, 9 dogs showing the fall, while 5 exhibiting a rise. Thus out of 8 cases with HAF of above 70 ml/min, 7 showed a fall in RHA by $31.7 \pm 15.6\%$. Out of 6 cases with HAF of below 70 ml/min, 4 showed a rise in RHA by $36.0 \pm 31.5\%$.

Twenty-nine dogs were injected ACh (3 µg) before and after exsanguination into the portal vein or hepatic artery and changes in RHA, RPV and RMA were observed (Table 1). As represented in Fig. 3, RHA showed a fall after intra-hepatic arterial administration (IHA), however, RPV rose transiently. RHA fell transiently after IHA in 27 cases out of 29. In the resting 2 dogs, however, peripheral resistance in the non-injected vessel changed in the same direction as that of the injected vessel. In other words, both simultaneously determined values of RHA and RPV showed an increase, regardless of whether ACh was given through IHA or IPV (Fig. 4). MABP was found always found to be below 50 mmHg in both the animals which displayed the above responses.

In 3 dogs, PV or HA were obliterated each for 1 min, and the elicited changes in the above mentioned values were observed. The obliteration of either PV or HA decreased the resistance of the other vessels (Fig. 5). After the obliteration of PV, RHA lowered by $25.3\%$ on the average. But after HA obliteration, RPV decreased by $6.91\%$ on the average (Fig. 5).

After PV obliteration, PVP fell immediately, lowering by $31.1\%$ from the control range of $157.5 - 227.5$ mmHg to $94.0 - 202.5$ mmHg. MABP began to fall from the
TABLE 1. Changes of peripheral resistances in the hepatic artery, portal vein and mesenteric artery after ACh (3 µg) injections intra-hepatic-arterially or intra-portal-venously

| Group  | No. of dogs | Ad. route of ACh | Peripheral resistance in vessels |
|--------|-------------|------------------|---------------------------------|
|        |             |                  | RHA Before | After | RPV Before | After | RMA Before | After |
| IHA    | 27          |                  | 1.265±0.428 | 0.859±0.286 | 0.047±0.024 | 0.073±0.035 | 0.548±0.294 | 0.637±0.386 |
| A      |             | IHA              | %Δ = −28.1±17.2 | %Δ = +65.5±59.9 | %Δ = +15.7±12.1 |
| IPV    | 2           | IHA              | 1.256±0.408 | 0.961±0.330 | 0.047±0.021 | 0.062±0.033 | 0.537±0.244 | 0.587±0.264 |
|        |             | IPV              | %Δ = −21.3±16.3 | %Δ = +33.3±27.3 | %Δ = +12.4±11.4 |
| IHA    | 2           |                  | 0.788 | 0.838 | 0.059 | 0.065 | 0.378 | 0.418 |
| B      |             | IHA              | %Δ = +6.6 | %Δ = +14.4 | %Δ = +4.7 |
| IPV    | 2           | IHA              | 0.732 | 0.825 | 0.060 | 0.077 | 0.356 | 0.418 |
|        |             | IPV              | %Δ = +12.2 | %Δ = +22.8 | %Δ = +15.6 |

Group A: the dogs showing MABP>50 mmHg, Group B: the dogs showing MABP≤50 mmHg, IHA: intra-hepatic-arterially, IPV: intra-portal-venously, %Δ: %change between Before and After.
control level of 107.5–117.5 mmHg, gradually attaining the mean of 98.3 mmHg shortly before the removal of obliteration. Thus the decrease rate was 12.6%. HAF increased by 23.4 ml/min on the average from the control range of 82.5–130 ml/min to 97.5–162.5 ml/min at the maximum. The peak of HAF was attained at about 20 seconds after the obliteration, and thereafter a gradual fall occurred. The value immediately before the release was, however, still higher than the control value by 9.2 ml/min on the average.

After HA obliteration, MABP rose immediately, being elevated from the control range of 105.0–120.0 mmHg to 120.0–132.5 mmHg. Thus the increase rate was 12.3%. PVP showed hardly any change. PVF increased from the control range of 291.7–308.4 ml/min to 316.7–325.1 ml/min at the highest. Thus the increase was 20.8 ml/min on the average.

Changes in MABP, blood flow and vascular resistance were observed in 3 dogs after decreasing PVF by shunting (Fig. 6). Immediately after the Sigma motor pump was set in motion, RPV began to rise, finally attaining a plateau. The maximum value was about 30% higher than the control, and the plateau level averaged 10%. At this time, RHA began to fall a little later than RPV, and then after a transient rise, it returned to the initial level.

Before and during the motion of the pump, MABP was kept at 70.0–132.5 mmHg, the average being 105.0 mmHg. PVP began to fall immediately after the start in motion of the pump, lowering from the mean control of 177.5 mmHg to the plateau level of 153.3 mmHg on the average. HAF, after the pump was started in motion, increased by 10.1 ml/min from the control level of 88.3 ml/min to the peak. Decrease in RHA started 18.7 sec on the average after increase in RPV. As seen in Fig. 6, there was an interval of about 20.0 sec between the peak of RPV and the cross point to zero-level of diphasic curve for the change in RHA.

**DISCUSSION**

Neural control of hepatic blood flow has long been a matter of discussion. Concerning its mechanism, however, physiological and anatomical views vary from one worker to another, and no consensus has been established. This is no doubt attributable to the complicated vascular system of the liver.

Bauer et al. (10), Brauer et al. (11), and Andrews (12), performed the experiments on the removed liver, and and Green et al. (13), Condon et al. (1), Shoemaker (2), Hanson and...
Johnson (3), and Takeuchi et al. (4) made observations on the denervated liver from dogs. The first report which disclosed the importance of hepatic nerve plexus was made by Francois-Frank and Hallion (14), who observed that the stimulation of the visceral nerve elevated the HAP. In 1910, Burton Opitz (15) reported that the stimulation of hepatic nerve plexus decreased HAF, but did not affect PVF irrespective of the presence or absence of a connection between the liver and central nervous system. Later, Takeuchi (16) again emphasized the importance of the hepatic nerve plexus in dogs, but few except for Cohn and Koutz (17), Kawazoe (7) and Wong (8) succeeded in experiments with intact hepatic nerve plexus.

The author's collaborators, K. Kawazoe and Z. Wong, made serial studies on the distribution of adrenergic and cholinergic receptors on the circulation beds of HA and PV of dogs. The bubble flowmeter method (9) they used to prevent the drop of perfusion pressure, resulting from resistance increase in the extracorporal shunt, or to avoid a certain fall in systemic blood pressure owing to bleeding, proved inadequate. In the present experiment, the use of the electromagnetic flowmeter, together with a cannulation type probe, made it possible to eliminate to some extent, the above mentioned defects. Moreover, in the present work, the gastroduodenal artery was ligated after branching from the proper hepatic arteries and the distal region was perfused with blood from the left femoral artery, thus possibly maintaining a more physiological state than when the gastroduodenal artery was merely ligated. It was confirmed that PVF increased by 20–30 ml/min over that seen after mere ligation of this artery.

As for the measurement of dog's HAF, Green et al. (13) gave 85.8±14.9 ml/min, Hanson and Johnson (3) 29.2±14.7 ml/min/100 g of liver, and Kikuchi (18) 346.2±142.8 ml/min. Suzuki (19), who used a non-cannulating type probe reported HAF of 30.5 ml/min/100 g of liver and PVF of 61.3 ml/min/100 g of liver at the start of observation, and HAF of 30.5 ml/min/100 g of liver and PVP of 54.7 ml/min/100 g of liver at one post-operative hour. In the present experiment, HAF was 82.7±22.9 ml/min and PVF 251.7±99.6 ml/min, which is in good parallel with the results by the above mentioned workers. The present results are not essentially different from the measurements in rats, as reported by Grayson and Mendel (20).

There have been many observations (7, 8, 16, 21–23, 25) on the distribution and the properties of cholinergic receptors on the circulation beds of HA and PV. Kawazoe (7), who administered 0.3–3.0 µg of ACh, published a view that a small number of vasodilating cholinergic receptors might be distributed in HA, and vasoconstrictive cholinergic receptors in PV. The exact opposite observation was however, sometimes obtained with dogs, depending on the experimental conditions. On the other hand, the responses to adrenaline and isoproterenol are highly stable. From these results, Wong (8) inferred the possibility that the relationship between the two vascular systems might be dependent on the MABP. Thus he estimated that some conditions such as a voluminous bleeding, and induced hypotension, might produce these abnormal vascular responses. This is a view of great interest because the underlying assumption seems to be that cholinergic receptors, distributed on the hepatic
vascular wall, are responsible not only for the constriction and the relaxation of the vascular smooth muscle but also for the communication of information between the two vascular systems. The finding in the present experiment that when the systemic blood pressure was below 50 mmHg, an atypical response was induced by ACh administration in the non-injected vessel is in good agreement with Wong's observation. It is, however, necessary to mention that even in these cases, the response within 1 min after ACh administration was hardly different from the ordinary typical response observed. Fischer (25) pointed out that ACh injected into PV did not act on PVF and MABP. In order to explain the role of PV in the hepatic circulation, he had advocated the "detoxification" hypothesis on the ACh-action in agreement with Green et al. (13). The discrepancy between their results and present ones was considered to be due to the differences in the mechanism of the flowmeters used and in MABPs for the dogs.

In order to elucidate the mechanism of appearance of this diphasic reaction, the obliteration of HA and PV and the PV shunting were carried out. It was confirmed that change in blood flow or in peripheral resistance in one of the two vessels always produces diphasic change in the resistance of the other. More important is the fact that the behavior of RHA response to change in the systemic pressure and in HAF was different depending on whether HAF was above or below 70 ml/min, which nearly corresponds to the systemic pressure of 90 mmHg as calculated from the relationship obtained from the present data. Consequently, when ACh is given through the IHA route to dogs with a systemic pressure of over 90 mmHg, RHA is considered to decrease slightly or to be constantly maintained, as shown in the present report. At the same time, the increase of RPV is ascribed to a reflex in the rise of RHA, which is never considered the direct action of ACh in the perfused blood, since the increase of RPV after IHA administration is always greater than that after IPV administration. But when ACh is given into the hepatic artery of dogs with a systemic blood pressure below 50 mmHg, fall of the systemic blood pressure and decrease of HAF, produced by the circulating ACh will have a dualistic effect in RHA, and as a consequence, a paradoxical phenomenon seems to be exhibited at 3 min and later following the ACh administration. It is assumed moreover, that the present results in the vessel obliteration experiment and in the PV-shunting one provide evidence that the intra-vascular administration of ACh can often produce a diphasic response even when MABP is in the 90-50 mmHg range.

The present results also provide an explanation for the diversity of views concerning the distribution of cholinergic receptors and suggest the possibility that a certain intervention existing between the responses of the two vascular system may be mediated by ACh. Grayson and Mendel (20), Hanson and Johnson (3), Greenway and Oshiro (24) and Takeuchi et al. (4) have discussed the communication system between HA and PV, however, the general physiological viewpoint has not been adequately covered. The methods used, the results obtained and the conclusions drawn were not in good agreement. The present report will be followed up by studies using various blocking agents such as hexamethonium or atropine.
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