STUDIES ON THE MICROCIRCULATION UNDER THE INFLUENCE OF THE AUTONOMIC NERVE IRRITATION

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Although it has been known that dysfunction of the autonomic nerve may be held responsible for causing a variety of disease states, it was by J. Reilly in 1934 that a convincing experimental evidence was presented for the fact that irritation of the autonomic nerve gives rise to an unspecific type of reaction within the organism with widespread pathological processes. Ever since, a large number of experimental studies have demonstrated that irritation of the autonomic nerve induces lesions not only in the organs which are under direct innervation of the nerve but also in those organs which are not directly related to the nerve. This phenomenon has been termed Reilly's phenomenon (Syndrome d'irritation neuro-végétative) (1), and has been the subject of a large number of clinical as well as experimental studies(2). The exact mechanism of this phenomenon, however, remains to be elucidated. The lesions induced as the result of autonomic nerve irritation include petechial hemorrhage in the pancreas, adrenals, myocardium, small hemorrhage and infarction in the lungs, scattered small hemorrhages in the gastric mucosa and hematuria. Those lesions are characterized histologically by marked dilatation and stagnation of the capillaries and petechial hemorrhages. This fact is in support of the view that Reilly's phenomenon is mediated by disturbance of the function of the vascular system.

Yamaguchi et al(3) has previously expressed the view that both autonomic nervous and endocrine systems are participating in the reaction to autonomic nerve irritation. The present study has been undertaken to investigate the behavior of the peripheral vascular bed in response to autonomic nerve irritation in order to obtain a clue for elucidating the complex relationship between those two systems.
Healthy male rats of donryu strain weighing between 180 and 200 g and healthy male white rabbits weighing around 2 kg were used as experimental animals.

Electric stimulation of the vagal and sympathetic nerve of the neck was utilized as means of irritation of the autonomic nerve. The animal (either the rat anesthetized by subcutaneous injection of thiopental sodium in the amount of 2 mg/kg of body weight or the rabbit anesthetized by intravenous injection of 12 to 15 mg/kg of the same substance), was placed in a spine position on a board. After shaving and anesthetizing the skin of the neck with an adequate amount of 0.5% procaine hydrochloride, a midline incision was made on the neck and the left vagal nerve trunk or the left sympathetic nerve trunk was exposed. A platinum electrode was hooked to the exposed nerve at the level of the carotid bifurcation, and rectangular impulse (30 V., 5 m.s., 50 c.p.s.) was delivered to the nerve three times, each time for a period of 30 seconds, with 10 seconds interval.

Microscopic observation method of the terminal vascular system of the mesentery originally advocated by Zweifach et al(4) and modified by Yamaguchi et al(5) was utilized for the study of the behavior and permeability of the terminal vascular bed: the mesentery of the animal was exposed and observed under a phase contrast microscope while 4% solution of pontamine sky-blue in the amount of 5 cc for the rabbit and 0.5 cc for the rat was injected through a polyethylene cannule inserted into the femoral vein or directly into the jugular vein and the degree of vascular permeability was evaluated on the basis of the intensity of staining by the dye of the connective tissue around the vessels.

RESULTS OF EXPERIMENTS


When the autonomic nerve is irritated while the mesenteric terminal vascular bed is visualized under the phase contrast microscope by means of the technique described above, it is observed that permeability of the wall of the terminal vessels increases regardless of whether the irritated nerve is orthosympathetic or parasympathetic in type. On the other hand, the pattern of blood flow through the terminal vascular network exhibits a slight difference when irritation was given to the vagal nerve as compared to that seen when the sympathetic nerve is irritated:

1) When the vagal nerve is irritated, blood flow through the terminal vascular network slows for an instant about 45 seconds after termination of irrita-
tion, only to resume an even faster circulation after several seconds. This finding may be interpreted as the result of an increased vasomotion of arterioles, metarterioles and venules. After a period of approximately five minutes, however, anastomoses between arterioles and venules open, venules become congested and cessation of blood flow ensues.

2) When irritation of the same intensity is given to the sympathetic nerve, on the other hand, no initial slowing of blood flow is observed, and a remarkable increase of vasomotion is seen from the beginning, and at about 30 seconds after termination of irritation blood flow suddenly slows, arteriolo-venular anastomoses open, congestion of venules and finally cessation of blood flow ensues.

2. The Effect of Various Agents upon the Modification of Vascular Permeability Induced by Autonomic Nerve Irritation.

A polyethylene canule was inserted through the left femoral artery into the abdominal aorta of the rabbit up to the level of the superior mesenteric artery, and 5 mg of prednisolone, 8 units of ACTH, 2 mg of DOC-glucoside or 6 mg of chlorpromazine was injected slowly. Immediately after injection, the vagal nerve of the neck was irritated by means of electric impulse and subsequent changes of the behavior and permeability of the mesenteric terminal vascular bed was investigated microscopically according to the technique described above. The results are as presented in Table 1. In summary it is observed that prednisolone

<table>
<thead>
<tr>
<th>Procedure</th>
<th>No. of animals</th>
<th>Vascular permeability</th>
<th>The behavior of the microcirculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irritation of the* left cervical sympathetic n.</td>
<td>5</td>
<td>+</td>
<td>Initial acceleration, followed by slowing down and arrest of blood flow</td>
</tr>
<tr>
<td>Irritation of the* left cervical vagal n.</td>
<td>5</td>
<td>+</td>
<td>Initial slowing down, then acceleration and again slowing down and arrest of blood flow</td>
</tr>
<tr>
<td>Irritation of the vagal n. + Prednisolone</td>
<td>5</td>
<td>−</td>
<td>Little change in blood flow. Vascular tonus well maintained</td>
</tr>
<tr>
<td>Irritation of the vagal n. + DOC-Glucoside</td>
<td>5</td>
<td>+</td>
<td>Little change in blood flow</td>
</tr>
<tr>
<td>Irritation of the vagal n. + ACTH</td>
<td>5</td>
<td>#</td>
<td>Vascular dilatation, arrest of blood flow, plasma skimming and red cell aggregatioin</td>
</tr>
<tr>
<td>Irritation of the vagal n. + Chlorpromazine</td>
<td>5</td>
<td>−</td>
<td>Little change in blood flow. Vascular tonus somewhat lost</td>
</tr>
</tbody>
</table>

* Rectangular impulse, 30 V., 5 m.s., 50 c.p.s., 30°×3

and chlorpromazine counteract the effect of vagal irritation upon permeability and vasomotion of the terminal vascular bed, whereas the same effect is
potentiated by ACTH and DOC-glucoside, to a greater extent by the latter. It is also noted that in the group of animals which was given ACTH intraarterially, the alteration of vasomotion and the pattern of blood flow is more pronounced than in the control group whereas in those animals which received DOC-glucoside little alteration is observed in vascular motility but a remarkable increase in vascular permeability occurs.

3. Studies on Adrenalectomized Rats.

A series of experiments was performed on groups of rats which were adrenalectomized in the following manner: under anesthesia by intraperitoneal injection of thiopental sodium, the retroperitoneal space was reached on each side of the spine through incisions, about 1 cm in length, along the dorsal costal margin, and the adrenals were exposed and removed.

<table>
<thead>
<tr>
<th>Table 2. The influence of various agents upon the microcirculation of the adrenalectomized rat</th>
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<tbody>
<tr>
<td>Agent</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Control (Adrenalectomy)</td>
</tr>
<tr>
<td>Prednisolone</td>
</tr>
<tr>
<td>ACTH</td>
</tr>
<tr>
<td>Epinephrine</td>
</tr>
<tr>
<td>Norepinephrine</td>
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<tr>
<td>DOC-Glucoside</td>
</tr>
</tbody>
</table>

Twenty-four to 48 hours after adrenalectomy, mesenteric terminal vascular bed was examined microscopically by the same technique as in the previous experiments. The results obtained were as follows:

1) Twenty-four hours after adrenalectomy, permeability of the terminal vessels is increased, blood flow in the vessels is slow and the tonus of the vessels is decreased. On the other hand, vascular permeability and vasomotion are normal at 48 hours after adrenalectomy.

2) In the experiments in which 0.2 mg of prednisolone, 0.15 mg of DOC-glucoside, 0.1 to 0.2 cc of 1:10,000 solution of epinephrine, 0.1 cc of 1:10,000 solution of nor-epinephrine or 0.4 units of ACTH was injected into the jugular vein of the rat which had been adrenalectomized 24 hours previously, the follow-
ing results were obtained: prednisolone counteracts the vascular permeability increasing effect of adrenalectomy and admirably restores the tonus of vessels and blood flow in the microcirculatory system to normal. DOC-glucoside intensifies the increase of vascular permeability induced by adrenalectomy, while restoring normal pattern of blood flow as prednisolone does. After injection of epinephrine, a marked alteration of the pattern of blood flow is observed, namely, a temporary retardation followed by acceleration, as well as opening of the arteriovenular anastomoses and some increase in vascular permeability occur. The effect of norepinephrine injection is similar to that of epinephrine but in a milder degree.

Injection of ACTH is immediately followed by a loss of vasomotion of the entire terminal vascular bed, resulting in dilatation of vessels and arrest of blood flow. Changes in vascular permeability after ACTH could not be assessed because the injected dye did not reach the terminal vascular bed.

4. Experiments on Hypophysectomized Rats.

In order to rule out the possibility that the effect of autonomic nerve irritation upon the microcirculation might be mediated by the vasodilating effect of the substance secreted from the pituitary gland, the behavior of the terminal vascular bed was studied in the hypophysectomized animal. The following experiments were performed on a group of rats which were hypophysectomized 3 days previously by the technique advocated by Koyama(6).

<table>
<thead>
<tr>
<th>Procedure</th>
<th>No. of animals</th>
<th>Increase in vascular permeability</th>
<th>The behavior of the microcirculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left cervical vagus irritation</td>
<td>3</td>
<td>−</td>
<td>Transient slowing down of blood flow</td>
</tr>
<tr>
<td>Left cervical symp. n. irritation</td>
<td>3</td>
<td>±</td>
<td>Transient acceleration of blood flow</td>
</tr>
<tr>
<td>ACTH I.V.</td>
<td>3</td>
<td>−</td>
<td>Blood flow initially slows down and stops, then becomes active</td>
</tr>
<tr>
<td>STH. I.V.</td>
<td>3</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Pituitran. I.V.</td>
<td>3</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Epinephrine I.V.</td>
<td>3</td>
<td>−</td>
<td>Initial acceleration, then slowing down, then again acceleration of blood flow. Operating of A-V anastomoses</td>
</tr>
<tr>
<td>Nor-epinephrine I.V.</td>
<td>3</td>
<td>−</td>
<td>Acceleration of blood flow</td>
</tr>
</tbody>
</table>
1) The mesenteric terminal vascular bed was examined microscopically in the hypophysectomized rat at 3 days after the operation, and it was observed that the pattern of blood flow as well as permeability of the terminal vessels did not show a remarkable difference from normal.

2) In response to the left cervical vagal nerve irritation, the terminal vascular bed of the hypophysectomized rat exhibited a very little increase in permeability and a modification of blood flow which was evanescent and mild in degree.

3) In response to irritation of the left sympathetic nerve trunk, the hypophysectomized rat also exhibited a modification of permeability and of the behavior of the mesenteric terminal vascular bed which was much less pronounced than in the group of animals with their intact pituitary glands.

4) Groups of hypophysectomized rats were injected intravenously with 0.4 units of ACTH (manufactured by Armor Co.), 1 rat-unit of STH (manufactured by Parke-Davis Co.), 0.1 cc of Pituitran (Pitressin manufactured by Shionogi Co.), 0.2 cc of 1:10,000 solution of epinephrine or 0.2 to 0.4 cc of 1:10,000 solution of norepinephrine. The results of microscopic examination of the mesentric microcirculation of those groups of animals were as follows; a slight increase in permeability of the terminal vessels is produced by injection of STH. and Pituitran, but not by other substances utilized. In response to injection of ACTH, STH and Pituitran, blood flow slows or stops evanescently but soon resumes a normal or even more active pattern of circulation. After injection of epinephrine, blood flow is acceleratd for a while in the begining, then gradually slows and finally becomes very rapid again. At the final stage, the arteriolo-venular anastomoses open and true capillaries become bloodless. Norepinephrine injection entails only acceleration of blood flow.

5. Relationship between the Modification of Mesenteric Microcirculation and Blood Pressure in the Large Artery.

A canule was inserted into the left femoral artery of the rabbit and changes of blood pressure in the vessel were recorded on a kinograph with the aid of a mercury manometer, while microscopic examination of the mesenteric microcirculation was carried out simultaneously. The results obtained were as follows:

Blood pressure in the artery falls during irritation of the left cervical vagal nerve and returns to normal after termination of irritation with or without a phase of rebound rise above the normal level. This pattern of changes of arterial blood pressure is unaffected by intravenous injection of prednisolone, chlorpromazine, DOC-glucoside or ACTH. However, the behavior of the mesenteric
terminal vascular bed shows very little modification while the fall in arterial blood pressure is manifest, except after injection of ACTH which produces a considerable change in the behavior of the microcirculation.


Through a median incision of the abdominal wall, a part of the ileum of the rat was pulled out of the peritoneal cavity onto a board and was covered with pieces of cotton soaked with 1% gelatin-Ringer's solution at 40°C. Thereafter irritation was given to the left cervical vagal nerve trunk as in the previous experiments. After five minutes, the mesenteric small vein was severed and red cell count was made on the blood sample obtained from the vein in order to study the modification of hemoconcentration in the vein in response to the autonomic nerve irritation. The results obtained are presented in Table 1. It is observed that hemoconcentration is evident in the group of animals which were given irritation to the autonomic nerve. In other groups of animals 0.4 mg/kg of Prednisolone, 0.4 units of ACTH or 0.1 mg/kg of DOC-glucoside was given three times starting from 24 hours previous to irritation of the autonomic nerve and modification of the red blood cell count of the mesenteric veni blood was compared with respective control groups which received the injection alone. As is shown in Text-figure 2, hemoconcentration in response to autonomic nerve irritation develops in the animal given ACTH, but not in animals given Prednisolone, and hemodilution in a slight degree was produced by combination of DOC-glucoside injection and autonomic nerve irritation. Similar experiments on adrenalectomized and hypophysectomized animals showed that little difference was observed between the groups with and without autonomic nerve irritation.
DISCUSSION

Chambers, Zweifach and their associate(7) have studied the architecture of the capillary system of vessels extensively, and it is now known that the system consists of a complex network of metarterioles, true capillaries, thoroughfare channels and metarteriolar-venular anastomoses. Of these structural elements, metarterioles and metarteriolar-capillary junctions (precapillary sphincters) are known to be provided with smooth muscle capable of contracting and dilating. It is evident from numerous studies that the contractility of the smooth muscle is under autonomic nervous control, and Pellegrini(8) demonstrated that mild (physiologic) stimulation of the vagus or the sympathetic entails opposite functional modifications of the abdominal small vessels, namely vasodilatation, slowing of blood flow and closing of arteriolo-venular anastomoses by vagus stimulation; vasoconstriction with ischemia, acceleration of blood flow and opening of arteriolo-venular anastomoses by stimulation of the splanchnic nerve. In the present study, using stimulation in a greater intensity (Irritation) of the autonomic nerve, it was shown that modifications of the behavior of blood flow in the terminal vascular bed in response to vagal and sympathetic irritation was different only in the initial phase and terminated in the same picture, namely opening of A-V anastomoses, congestion of venules and cessation of blood flow. It was also shown that chlorpromazine inhibits the development of such modifications in response to autonomic nerve irritation. These facts are in accord with
the view held by Laborit(9) that irritation entails a oscillatory type of reaction in the body, which is called by him “Réaction oscillatoire postagressive,” and with the view held by Yamaguchi and others that this type of reaction can be counteracted, and the morbid state produced as the result of such a reaction can be minimized, by administration of chlorpromazine. Relatively little is known about the relationship between capillary permeability and autonomic nerve activity. Engel(10) postulates a permeability-controlling function for the sympathetic based upon experiments in which the vessels of the knee joint were perfused with a dye-containing fluid. In the present study, irritation of the autonomic nerve, whether sympathetic or vagal, produced a pronounced increase of permeability of the small vessels. It is noted, however, that manifestation of increased permeability—staining of the tissue around the vessel with the dye—appeared not only in true capillaries but also in venules. The exact nature of the factor, whether nervous or humoral, which controls vascular permeability, however, is yet to be determined.

Much more is known about the effect of adrenal cortical hormones upon capillary permeability. Swingle(11) observed an increase in vascular permeability in the adrenalectomized animal, and Menkin(12) found that adrenal cortical extract and cortisone inhibit the capillary permeability increasing activity of leucotaxin, whereas ACTH does not antagonize leucotaxin but does counteracts exudin in the effect upon capillary permeability. In relation to the effect of DOCA on vascular permeability controversial results have been obtained by different authors. Fine et al(13) have reported his observation that DOCA given intravenously produced a decrease of capillary permeability, whereas Freed(14) demonstrated increased capillary permeability after local application of DOCA. In the present study it has been shown that the increase in vascular permeability developing in response to vagal irritation is inhibited by prednisolone and chlorpromazine, and enhanced by ACTH and DOC-glucoside, to a greater extent by the latter substance.

In the previously reported experimental and clinical studies on the Reilly's phenomenon, it has been shown that cortisone inhibits and ACTH aggravates the development of pathological processes produced by autonomic nerve irritation. The results of the present study seem to give the evidence for the assumption that cortisone inhibits the development of the phenomenon by protecting the microcirculation from the effect of autonomic nerve irritation, whereas ACTH aggravates lesions in various organs by potentiating the effect of autonomic nerve irritation upon the microcirculation.

The results of experiments with epinephrine and norepinephrine show that,
judged from the behavior of the microcirculation, these agents do not seem to give a good effect upon maintenance of adequate tissue function, and the therapeutic effect of these substances in the condition of stress should be reexamined.

The comparison of blood pressure in the large artery with the behavior of the microcirculation under the effect of autonomic nerve irritation reveals that the modification in the former is a poor representation of the latter. This fact should be taken in consideration in dealing with the state of shock.

No reports have yet appeared in the literature on the effect of DOC-glucoside on Reilly's phenomenon. In the light of the present study, however, it can be postulated that this substance, like ACTH, has potentiating effect upon the effect of autonomic nerve irritation.

The results of experiments with STH and Pituitran in the present study are in accordance with the view held by Krogh(15) that the pituitary posterior lobe hormones have a controlling effect upon vascular endothelium and smooth muscle.

The question of how much are the results obtained in the present study applicable to the behavior of the microcirculation of other organs is yet to be determined. Also undecided is the exact mechanism of the action of autonomic nerve stimulus upon the microcirculation. In this respect it will be of great interest to investigate the effect on the microcirculation and the relationship with the function of the autonomic nervous system of such substances as histamine, serotonin, leucotaxin, heparine and plasmine.

SUMMARY AND CONCLUSIONS

The change in the behavior and permeability of the mesenteric microcirculation of the rat and rabbit under the influence of the autonomic nerve irritation and endocrine factors was studied using the direct phase-contrast microscope observation method.

Irritation of the left cervical autonomic nerve, either vagal or sympathetic, resulted in extreme vascular dilatation, arrest of blood flow and increase in vascular permeability.

Intraarterially administered prednisolone and chlorpromazine inhibited, and ACTH enhanced, such an adverse effect of the autonomic nerve irritation upon the microcirculation, observed. DOC-glucoside increased vascular permeability in cooperation of the influence of autonomic nerve irritation as well as by itself, but exerted little effect upon the vasomotion of the microcirculatory system.

The mesenteric microcirculation of the rat 24 hours after adrenalectomy showed a loss of vascular tonus indicated by slow blood flow, and an increase in vascular permeability, which were rapidly restored to normal by administration
of prednisolone and chlorpromazine, administration of epinephrine or norepinephrine to the adrenalectomized rat caused a marked change in the pattern of blood flow through the microcirculatory system due to increased vasomotion and opening of the A-V anastomoses. In the hypophysectomized rat, the autonomic nerve irritation caused little alteration in the behavior and permeability of the microcirculation. ACTH and posterior pituitary preparations induced a slight dilatation of the terminal vessels in the hypophysectomized rat.

Simultaneous recording of the femoral artery pressure and the behavior of the mesenteric microcirculation of the rabbit indicated that the changed in the former was a poor indicator of the change in the latter.

On the basis of these findings, it is concluded that the integrity of the microcirculation is maintained by the intimate coordination of the autonomic nervous and pituitary adrenal control, and the disturbance in either one of the two systems results in the loss of appropriate function of the microcirculation, thence in disturbance of tissue homeostasis.

It is emphasized that the behavior of the microcirculation should be taken into consideration in dealing with the various clinical conditions in which the disturbance of the autonomic nervous system apparently plays an important role.

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BIBLIOGRAPHY

Fig. 1. Microcirculatory system of the rat mesenterium before irritation of the left cervical vagal nerve trunk visualized under long focus phase contrast microscope. (Magnification x 175)

Fig. 2. Transitory vascular dilatation immediately after irritation of the left cervical vagal nerve trunk. (x 175)

Fig. 3. Vascular contraction. (x 175)

Fig. 4. Opening of A-V anastomoses. (x 175)

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Fig. 5. Blood flow through the terminal vascular bed is sluggish and the thoroughfare way is markedly dilated. (x 175)
Fig. 6. Blood flow through the thoroughfare way has completely stopped. (x 175)
Fig. 7. Erythrocyte aggregation is seen in the thoroughfare way. (x 175)
Fig. 8. Microthrombus and petechiae formation are noted. (x 175)

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