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Action of a Water Soluble Splenic Material on Anaesthetized Rat Blood Pressure

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The effects of a biologically active water-soluble splenic material on the rat blood pressure are described. The endovenous perfusion of the splenic material in acidified and alcalinized forms caused significant increases of the mean blood pressure in normal, vagotomized and pithed rats, showing that, in contradiction to previous reports, changes in pH did not affect its hypertensive activity. In normal rats, the hypertensive responses were not affected by the α- and β-adrenoceptor antagonists: tolazoline, ergotoxine and propranolol, supporting the previously stated view that adrenergic mechanisms are not involved. In addition, the aforementioned results obtained in vagotomized and pithed rats suggest a lack of involvement of cardiac and vascular nervous mechanisms.

Key words: Blood pressure, Splenic material.

Since GANDARIAS (6) obtained a water soluble material extracted from spleen, lung and pancreas of rats and rabbits with striking activity on smooth muscle, there has been a continued interest in this smooth muscle-active agent. Further experiments have shown a similar activity on smooth muscle with extracts of rabbit renal cortex (4) and bovine spleen (1, 2, 8, 10). Studies on this active material, mainly extracted from bovine splenic tissue, have provided considerable information about its biological activity (1, 2, 5, 7-11).

It has been stated that the water soluble splenic material promotes remarkable cardiovascular effects in cats and rabbits (6, 7, 11). However, there is little information on the cardiovascular actions of splenic material in other species. Therefore, the aim of the present work has been to study the cardiovascular effects of the water soluble splenic extract in rats under different conditions: normal, vagotomized and pithed states. An approach to the mechanism of action using alpha and beta adrenoceptor blocking agents has also been attempted.

Material and Methods

Fed male and female Wistar rats weighing between 250-450 g were used. The animals were anesthetized with urethane 25 % s.c. and i.p. (0.6 ml/100 g). Then, they were tracheotomized and their carotid artery dissected and cannulated. Heparin (200-500 units) was administered endovenously through a cannula fitted inside the femoral vein and the carotid cannula was connected to a pressure transducer. Blood pressure measurements were recorded in a Hewlett-Packard 7754A multi-pen recorder. To perform the experimental assays the animals were divided into three groups: the first one was the intact group; the second one was vagotomized by bilateral section of vagal trunks at cervical level; the third one was subjected to a pithing treatment by introduction of a metal pin, 2 mm in diameter and 25 mm in length, into the skull through the orbit and along the vertebral canal to destroy the spinal cord. This is a fit biological condition avoiding nervous influences in checking the effects of cardiovascular active substances. This group was maintained under mechanical respiration.

The extracts were obtained from fresh bovine spleen. The raw material was bruised and salted out by addition of 5 ml of 0.3 N NaOH and 5 ml 5 % $ZnSO_4$ solution (3) per g of wet tissue. The material was then homogenized in a MSE ultrasonic homogenizer (6 kHz × 3 min) and centrifuged at 5,200 *g* for 5 min. The precipitate was discarded and the supernatant collected. Portions of this stock extract, pH close to 7, were adjusted to the working pH values: 5 and 9, and then stored at 2-4°C. Under these conditions the extract remained active for at least one week. When lyophilized, the activity remained unchanged for several months.

The following drugs were used: propranolol hydrochloride (ICI), tolazoline hydrochloride (CIBA) and dihydro ergotoxine metoxylate (Sandoz). All drugs were dissolved in saline.

Extracts were administered for 10 min by continuous perfusion using a syringe pump at a flow rate of 0.15 ml/min (corresponding to 15 mg of fresh tissue per min). A period of 30 min was allowed for equilibration. Then blood pressure values were recorded for a minimum of 30 min to check for possible delayed effects.

Experiments (n = 6) carried out in order to determine the influence of the chemicals used in the treatment of the splenic material during the extraction procedure showed that there were no significant changes in the basal blood pressure values before and after addition of the pooled extracting reagents, under the same pH conditions as the splenic extract.

The experimental procedure in the assays with adrenoceptor antagonists is described in the results.

The results are expressed as means ± SE mean for the number of the experiments indicated *n*. Significant differences between means were calculated by Student's paired t test.

Results

The administration of splenic extract caused striking increases in mean blood pressure of normal rats (table I). Both alcalinized and acidified extracts caused similar increases in blood pressure indicating that changes in pH did not significantly affect the activity of the splenic material.

In vagotomized rats, the splenic extract induced hypertensive responses close to those of normal rats (table I). The acidified extract caused as much increase in blood pressure as the alcalinized form. As in normal rats, variations in pH did not involve significant changes in the hypertensive responses.

Table I. *Effects of splenic extracts (1.50 ml/10 min) on the mean blood pressure of anaesthetized rats under different treatments and normal rats treated with alpha and beta adrenoceptor antagonists.*

Each value, expressed as the mean ± S.E.M. for the number of experiments (n), indicates the variation of the blood pressure, mm Hg, with respect to the correspondent mean basal blood pressure. Statistical significancies are referred to basal pressure levels reached in each condition including the case of adrenoceptor blocking agents in which the mean values of variation are referred to the basal pressure levels attained before the administration of he blocking agents.

Treatment	n	Δ Blood pressure (mm Hg) Alcalinized S.E.	Acidified S.E.
Normal	6	35.32 ± 6.38**	39.70 ± 7.71**
Vagotomized	6	41.66 ± 8.16**	46.50 ± 7.60**
Pithed	6	33.33 ± 6.05**	34.16 ± 3.76**
Adrenoceptor antagonists		None	Acidified S.E.
Tolazoline (2 mg)	5	— 19.00 ± 5.41**	47.40 ± 6.80**
Ergotoxine (0.06 mg)	5	— 16.00 ± 2.09**	59.00 ± 6.51**
Propranolol (0.2 mg)	5	— 12.00 ± 4.54*	49.00 ± 7.41**

* $p < 0.05$; ** $p < 0.01$.

When the assays were performed in pithed rats the mean value of spontaneous blood pressure was considerably lower than that of normal and vagotomized groups, as it is well known. The administration of extracts caused a significant but transitory rise in blood pressure that was followed by a fall to basal or lower levels, even when the perfusion was being performed. Both alcalinized and acidified extracts caused similar effects. In the assays with the alpha and beta adrenoceptor antagonists, they were administered to normal rats 90 s before the perfusion of the extracts, in doses of 2 mg of tolazoline, 0,06 mg of ergotoxine, that suppressed the effects displayed by norepinephrine in concentration of 10^{-6} M that gave similar responses to those of the extracts, and 0.2 mg of propranolol. All these adrenoceptor blocking agents caused a slight fall in blood pressure (table I). The perfusion of acidified splenic extract, performed separately in groups of experiments for each antagonist, caused striking increases in blood pressure. The mean values of blood pressure increases referring to the basal levels before the administration of the blocking agents are shown in table I. After the perfusion all three values returned to basal levels.

Discussion

Although splenic extracts have sometimes been used in therapeutics, their pharmacological activity, particularly with respect to the activity on smooth muscle was relatively unknown. In 1948 SALVA (13) made reference to the therapeutic use of water soluble splenic extracts in some forms of intestinal atony, and later KRONTIL and KLABUSAY (12) described a vasodilator and hypotensive effect of the splenic extracts in several species. This effect was blocked by atropine and other similar agents. However, the splenic extract obtained by GANDARIAS (6) preserves its effectiveness in the presence of

atropine (1, 2, 7, 8, 10, 11). In addition the activity on vascular smooth muscle of cats and rabbits was not blocked by alpha and beta adrenoceptor antagonists (6, 7, 11). In this paper a more detailed characterization of the cardiovascular effects of the splenic extracts has been attempted.

The results, in normal rats, show that the alcalinized splenic material caused a hypertensive response, which was in agreement with that previously stated (6, 7, 11) in cats and rabbits. Conversely, the increase in blood pressure elicited by the acidified extract was in contradiction to previous reports (6, 7, 11) describing hypotensive responses in cats and rabbits. This discrepancy may be related to the different species of animals used and/or to the different extract administration procedure.

As has been previously described (6, 7, 11) the increasing blood pressure responses were not significantly affected by alpha and beta adrenoceptor antagonists. These results provide evidence against the involvement of alpha adrenoceptors in vascular smooth muscle and beta adrenoceptors in cardiac muscle, in the actions displayed by the splenic extracts.

There are no prior descriptions on the effect of the splenic extract in vagotomized and pithed states. As can be seen in the results, the effects induced by the extracts in both vagotomized and pithed situations were not different from those of normal rats, suggesting the absence of any cardiac and vascular nervous reflexes in the mechanism of action of these extracts.

From the results it can be concluded: both alcalinized and acidified splenic extracts administered by endovenous perfusion have a hypertensive effect on vascular smooth muscle of normal, vagotomized and pithed rats; the hypertensive response, in normal rats, remains without significant changes after treatment with alpha and beta adrenoceptor blocking agents, suggesting that adrenergic mechanisms are not involved. This supports the view stated in previous works (1, 2, 7, 8, 10, 11) that the smooth muscle stimulating effect produced by splenic extracts was not mediated by adrenoceptors, cholinoceptors and 5-hydroxy-tryptamine receptors. However, some evidences supporting that H_1- histamine receptors may be involved in the contractile response of some smooth muscle preparations to the splenic extracts, have been described (2, 10). The hypertensive response to splenic extract was maintained after suppression of cardiac and vascular nervous reflexes by vagotomy and pithing treatment respectively, suggesting that these nervous mechanisms are not involved.

Further experiments are needed to improve the knowledge on the chemical nature and the mechanism of action of this water soluble splenic material with striking activity on vascular smooth muscle.

Resumen

Se estudian los efectos de un material esplénico hidrosoluble sobre la presión arterial de ratas normales, vagotomizadas, demeduladas y tratadas con bloqueantes de α y β adrenoceptores. En todos los casos, la administración del material esplénico, mediante perfusión endovenosa, provoca incremento de la presión arterial que no resulta afectado por vagotomía, demedulación ni tratamiento con bloqueantes adrenérgicos. Las respuestas hipertensoras obtenidas al perfundir el material esplénico, tanto en forma acidificada como en forma alcalinizada, fueron similares, lo que, en contraste con descripciones precedentes, indica que su actividad sobre presión arterial de rata no resulta alterada por variaciones del pH.

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