Distribution of the Blood Flow Supplied by the Vertebral Artery in Rats: Anatomical, Functional and Pharmacological Aspects[1]

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Summary. In rats, the vertebral artery makes only a minor contribution to the blood perfusion of the ponto-medullary area. This was measured with radioactive microspheres and was confirmed by methylmetacrylate casts and local injection of a centrally acting hypotensive drug.

In numerous studies, the central hypotensive action of drugs has been evaluated by local injections into the vertebral artery in rabbits[3], cats[4,5] or dogs[4,6,7]. The distribution of the vertebral blood has been studied in rabbits[8], cats[9–12] and dogs[10–14] using various techniques such as the injection of radiopaque or dye solutions, of polymerizing compounds or of radioactive microspheres.

The present investigation deals with the distribution of materials injected into the vertebral artery of the rat, using polymerizing compounds and radioactive microspheres. The effect of a centrally acting hypotensive drug, α-methyldopa, administered by the same way was also studied.

Material and methods. 1. Functional and anatomical studies of the cerebral circulation. Adult male Wistar rats (400 to 500 g) were anaesthetized with 0.6 ml of Hypnorm® s.c. (= fluanison, 6 mg + fentanyl 0.12 mg). In all instances, a polyethylene catheter was advanced centrally into the left subclavian artery until the catheter tip reached the ostium of the vertebral artery without impeding the blood flow to this vessel. The same surgical procedure was followed as described below for renal hypertensive rats.

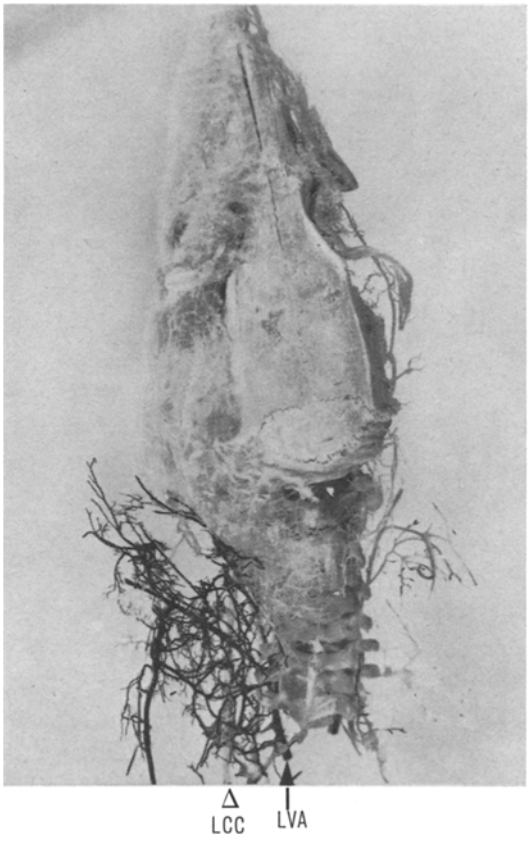

Fig. 1. Extracranial blood supply. Dorsal view on methylmethacrylate cast of the vertebral and carotid vascular beds of the rat. Photographs were taken with a red filter in order to differentiate between red and blue stained arteries. Dark (blue stain): vessels filled via the left vertebral artery (LVA). This concerns mainly the territory of the left-sided neck muscles. Light (red stain): vessels filled under equal pressure via the left common carotid artery (LCC). This concerns mainly the blood supply to the head.

In 5 experiments, radioactive microspheres of 15 ± 5 μm diameter (3M-Company) were infused through the catheter. The microspheres were suspended in 10% dextran and 0.5% Tween 80. A volume of 0.5 ml was infused over a period of 2 min. The spheres were labeled with ^{85}Sr. The injected amount of radioactivity ranged from 0.44 to 0.51 μCi. Approximately 5 min after the injection of the microspheres the animals were killed with an overdose of anaesthetic. The head and neck were fixed in 4% formaldehyde for 3 days. After this period the following tissue samples were taken: the whole brain (including cortex, cerebellum and brain stem), the left neck muscles, the right neck muscles, the remaining cervical structures from C_2 to C_7. The samples were counted and the radioactivity was calculated per gram tissue as previously described[10,12] and expressed as % of the injected amount.

In a second series of anaesthetized rats, in addition to the catheter to the left vertebral artery, another catheter was introduced peripherally in the left common carotid artery at the midcervical level. Simultaneously, polymerizing methylmethacrylate (Technovit, type 8001®) was infused through both catheters. The vertebral artery was filled with blue stained material, whereas through the left common carotid artery, the external and internal

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carotid beds were filled with red stained material. During the injection procedure the reflux to the aorta and to the heart was blocked by clamping at the base of both subclavian arteries. In 3 rats, 1 ml of coloured methylmethacrylate was infused in 2 min through either catheter. In 3 other rats the infusions were made under constant pressure, which was identical in both injection catheters, since both filling reservoirs were connected to the same expansion chamber at 200 to 300 cm H_2O. The casts were prepared as previously described[12].

2. Pharmacological studies performed in non-anaesthetized renal hypertensive rats. Renal hypertension was induced by application of a solid silver clip (internal diameter 0.20 mm) on the left renal artery of male Wistar rats (outbred stock, Cpb, WU, TNO, Zeist, The Netherlands) weighing 130–160 g[15]. The hypertensive rats were used, 4–6 weeks after application of the clip. Body weight range at that time was 200–240 g. A permanent indwelling iliac cannula was used for continuous recording of blood pressure[16]. Cannulation of the iliac artery as well as that of the subclavian artery were carried out under ether anaesthesia at least 1 day before the experiments were performed. The technique for injection of drugs into the vertebral artery[17] was modified to prevent respiration disturbances in conscious rats. Instead of a complete thoracotomy, only the clavicle and the first rib were transected in order to expose the left subclavian artery. A cannula (PP 10) was inserted into the left subclavian artery and ligated in such a way that its tip was laying at the bifurcation of the vertebral artery. After the implantation, the clavicle and the first rib were fastened to the sternum and muscles and skin were stiched. Aqueous solutions of α-methyldopa were infused in the unaesthetized rats via this catheter at a rate of 0.01 ml/min.

Results. Virtually no microspheres were found within the brain in any of the animals and the average radioactivity, expressed per gram tissue as % of the injected amount was 0.1% (range: 0 to 0.35). The corresponding average in the neck muscles on the injected side was 7% (range 4 to 12), whereas in the contralateral neck muscles it was 0.1% (range 0 to 0.38). Various amounts of microspheres were detected in the remaining neck structures (range 0.1 to 3.8%). These results indicate that the blood of the vertebral artery is mainly distributed extracranially and the greater part of it goes to the homolateral neck muscles. The plastic casts formed by methylmethacrylate filling of the left vertebral and carotid arteries confirmed these findings. In all cases the blue-stained material,

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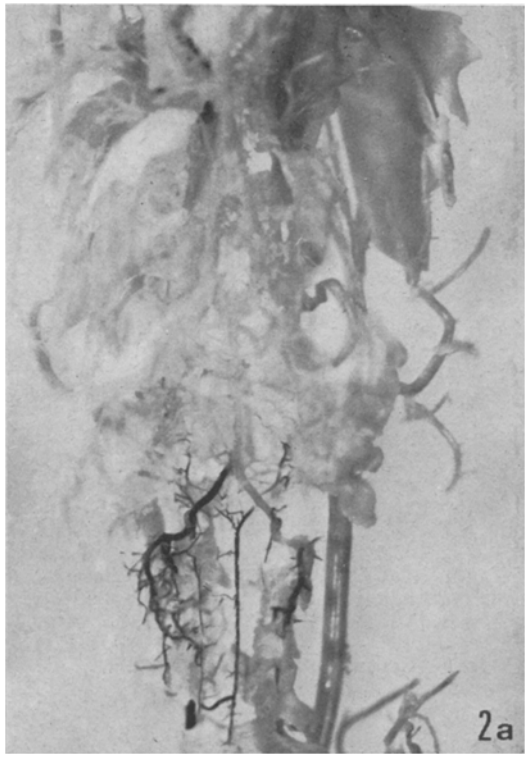

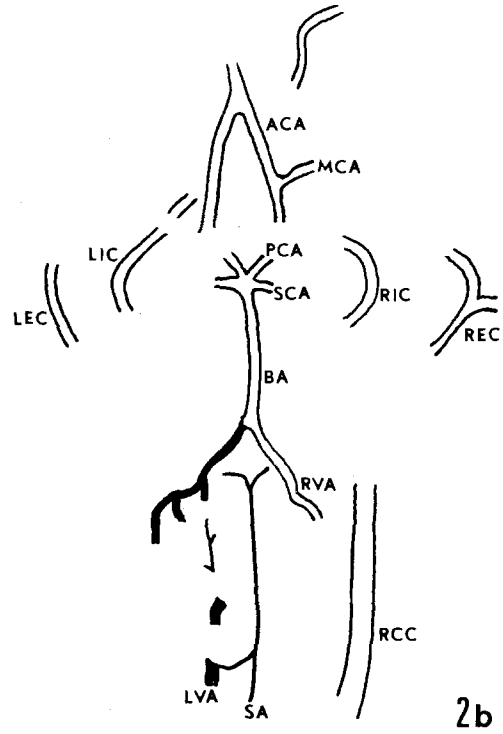

Fig. 2 a) and b). Intracranial blood supply.

a) Dorsal view on plastic cast of the vertebral and carotid vascular beds after withdrawal of the skull and of dorsal vertebral parts. Extracranial vessels were also largely removed in order to allow a better view on the intracranial vascular structures. Dark (blue stain): injection via the left vertebral artery, which is partly hidden behind vertebral parts out of focus. The left vertebral side branches and the spinal artery are clearly shown. The right vertebral artery is retrogradely filled with a mixture of blue and red material. Light (red stain): injection via the left common carotid artery, filling the whole vascular area of the circulus Willisii and the basilar artery and its side branches; retrograde filling of the right vertebral and carotid arteries.

b) ACA, anterior cerebral artery; RCC, right common carotid artery; BA, basilar artery; REC, right external carotid artery; LEC, left external carotid artery; RIC, right internal carotid artery; LIC, left internal carotid artery; RVA, right vertebral artery; LVA, left vertebral artery; SA, spinal artery; MCA, middle cerebral artery; SCA, superior cerebral artery; PCA, posterior communicating artery.

injected via the left vertebral artery, filled the vascular bed of the left neck muscles (Figure 1). In the basilar artery, particularly at its caudal end, a mixture of blue and red material was observed. Mixed material was also found in the territories which were retrogradely filled via the right vertebral artery.

However, the smaller vessels of the brain, even in the ponto-medullary area were filled with red-coloured methylmethacrylate coming from the carotid artery (Figure 2).

In conscious, renal hypertensive rats, administration of α-methyldopa by the i.p. route (10–800 mg/kg) caused a dose-dependent decrease in blood pressure (7–53 mm Hg). After infusion of α-methyldopa (25 mg/kg) into the jugular vein also a fall in blood pressure was observed. Blood pressure decreased gradually with a maximum 5 h after starting the infusion (-27 ± 5 mm Hg). The decrease in blood pressure after infusion of the same dose of α-methyldopa into the vertebral artery was less pronounced (-16 ± 3 mm Hg after 5 h). The difference, however, was not significant at 2–6 h. Intravertebral fusion of α-methyldopa in a dose of 10 mg/kg caused no change in blood pressure.

Discussion. The blood pressure lowering effect of α-methyldopa in the rat is mediated by a central action of the drug[16,18,19] and is still present after midcollicular transection of the brainstem[20]. In cats, and to a lesser degree in dogs, the major part of vertebral artery blood goes to the ponto-medullary structures of the brain[10–12]. In cats, the intravertebral injection of α-methyldopa lowers the blood pressure at doses which do not lower blood pressure after i.v. injection[21]. We found no differences between intravertebral and i.v. administration of a low dose of α-methyldopa in renal hypertensive rats. An explanation for this finding was provided since the radioactive microspheres, injected into the vertebral artery of the rat were mainly found in the neck muscles on the injected side and not in the brain. This means that drugs, when injected in a similar way, will arrive mainly in the extracranial tissues. Consequently, a centrally induced hypotensive effect of α-methyldopa cannot be demonstrated with this injection technique in rats. The quantitative findings of microsphere distribution were further confirmed by cerebro-vascular methylmetacrylate casts of the rat. In these casts, the sidebranches of the basilar artery providing the pontomedullary structures were mainly filled by material injected via the common carotid artery. It is concluded that injection of compounds into the vertebral artery of the rat is not an appropriate procedure for the evaluation of their pharmacological activity at the level of the rhombencephalic structures.

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Effect of (+)-Catechin on Renal and Intestinal Transport

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Summary. The ability of dog renal cortex slices to accumulate β-methyl-glucoside or glycine is enhanced by the flavonoid (+)-catechin at a concentration of 3.5 m*M*. This stimulatory effect is apparently due to a decreased rate of efflux of either substrate. On the other hand, the uptake of p-amino-hippuric acid and N^1-methyl-nicotinamide is inhibited by (+)-catechin. The drug at the same concentration is without action on amino-acid transport by guinea-pig intestine in vitro.

The therapeutic and pharmacological effects of flavonoids have been related to their action at the level of the vascular endothelium[2], and some of these effects may be a consequence of the interaction of these drugs with membranous structures.

Certain effects of flavonoids on membrane permeability have been studied. Tesi and Forssmann[3] have shown that the passage of inulin across isolated rat mesentery was inhibited by a soluble derivative of (+)-catechin, namely Na(+)-epicatechin-2-sulphonate. Ring et al.[4] demonstrated that the passive penetration of thiourea and amino-acids into bacterial and animal cells was reduced by (+)-catechin, whereas the polar derivative used in above-mentioned study had no effect. In the present paper, we report the effect of (+)-catechin on the active transport and accumulation of substrates in epithelial cells of renal cortex and small intestine in vitro.

Methods and materials. Studies on renal cortex slices: The experiments were performed on kidneys from healthy mongrel dogs. Much of the methodology closely follows that described by Robinson[5]. After removal of the renal capsule, the cortex was dissected and cut with a spring-loaded guillotine[6] into slices of 0.4 mm thickness. The uptake of β-methyl-glucoside, glycine, *p*-amino-hippuric acid (PAH) or N^1-methyl-nicotinamide (NMN) was determined after incubation of the tissue slices in a solution of the labelled substrates in Krebs bicarbonate buffer at 37 °C for 60 min. (+)-Catechin {(+)-cyanidanol-3} was added at a concentration of 3.5 m*M* to the appropriate solutions. After the incubation, the slices were briefly

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