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Autoregulation of renal blood flow during the infusion of acetylcholine or carbachol in anaesthetized dogs

NOBUYAKI OGAWA*, HIROSHI ONO, *Department of Pharmacology and Toxicology, Hatano Research Institute, Food and Drug Safety Center, 729-5, Ochiai, Hadano, Kanagawa, 257, Japan*

Whether renal blood flow autoregulation is abolished by acetylcholine was re-examined by kidney perfusion experiments in anaesthetized dogs. Renal blood flow was dose-dependently increased by the renal arterial infusion of acetylcholine (2 and 5 μg min^{-1}) or another muscarinic agent, carbachol (2 and 5 μg min^{-1}) and was maintained at an increased level during the infusion. When perfusion pressure was changed stepwise between 60 and 200 mmHg, the infusion of acetylcholine or carbachol caused no impairment of autoregulation. It is concluded that autoregulation is independent of muscarinic stimulation of vascular smooth muscle in the kidney.

Previous studies have shown that vasodilators, such as papaverine, nifedipine and verapamil, abolish renal blood flow autoregulation (Ono et al 1974; Hashimoto et al 1980). However, we recently demonstrated that not all the vasodilators influenced renal blood flow autoregulation. For instance, nitro-compounds which produce relaxation in smooth muscle through cyclic (c)GMP increase did not abolish the autoregulation (Ogawa & Ono 1986).

There are several biogenic substances that cause vasodilation. Previously, we reported that intrarenal infusion of prostaglandin E_2 or bradykinin had no effect on renal blood flow autoregulation, in spite of an obvious increase in renal blood flow (Ogawa & Ono 1985). The effect of acetylcholine on renal autoregulation has been controversial (Nahmod & Lanari 1964; Kiil et al 1969).

The aim of the present investigation has been to determine whether acetylcholine influences renal blood flow autoregulation. We examined the effect of acetylcholine together with another muscarinic stimulator, carbachol, on autoregulation of renal blood flow over a wide range of perfusion pressure in dogs.

Materials and methods

Eight mongrel dogs of either sex, 12–18 kg, were anaesthetized with α-chloralose (40 mg kg^{-1}) and urethane (400 mg kg^{-1}) intravenously, preceded by sedation with morphine hydrochloride (2 mg kg^{-1} s.c.). The left renal artery was exposed retroperitoneally, cannulated and perfused with blood conducted from the carotid artery by means of a Harvard peristaltic pump (Model 1215). An initial dose of 500 u kg^{-1} of sodium heparin was given as anticoagulant. Perfusion pressure was regulated by the use of a Starling's pneumatic resistance through which excess blood was conducted to the left jugular vein. A desired level of perfusion pressure was obtained by changing the pressure of the pneumatic resistance. Perfusion pressure and systemic blood pressure in the femoral artery were measured with an electric manometer (transducers: Statham P23Db and carrier amplifiers: San-ei 1206B). Renal blood flow was measured by an electromagnetic flowmeter (Narco RT-500). These parameters were recorded on an ink-writing oscillograph (San-ei 8S-53). When necessary, smaller doses of α-chloralose and urethane were supplemented, and sodium heparin was supplemented constantly at 100 u kg^{-1} h^{-1}. A drug solution was infused into a rubber tube connected close to the shank of the renal arterial cannula by the aid of an infusion pump (Harvard Model 901).

Drugs used in this experiment were acetylcholine chloride (Wako) and carbamylcholine chloride (carbachol, Sigma). Both were dissolved in 0·9% NaCl (saline), and the doses are expressed as the base.

The efficiency index of autoregulation (ARI) was calculated according to Semple & DeWardener (1959):

$$ARI = \frac{(RBF_2 - RBF_1)/RBF_1}{(P_{RA2} - P_{RA1})/P_{RA1}},$$

where the renal blood flow changes to RBF_2 from the initial value of RBF_1 when renal perfusion pressure is altered to P_{RA2} from the initial value of P_{RA1}.

Data were described as the mean ± s.e. Statistical analysis was according to the paired *t*-test and a difference with a *P* value of 0·05 or less was considered to be significant.

Results

Control observations usually confirmed excellent autoregulation of renal blood flow between 120 and 200 mmHg of perfusion pressure, and partial autoregulation between 100 and 120 mmHg (Fig. 1A, B). Renal blood flow autoregulation was not observed below 100 mmHg (Fig. 1A,B).

In 4 dogs, acetylcholine was infused into the renal artery at doses of 2 and 5 μg min^{-1}, and the blood flow change was observed in response to stepwise changes of perfusion pressure between 60 and 200 mmHg. The vasodilation reached a maximum 2 min after the onset of the infusion, and the renal blood flow sustained an

* Correspondence.

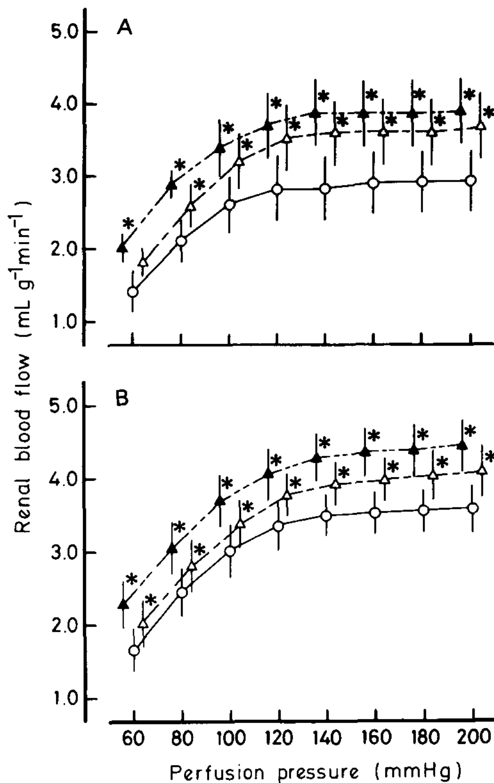

FIG. 1. Effect of (A) acetylcholine and (B) carbachol (○ control, △ 2 μg min^{-1}, ▲ 5 μg min^{-1}, n = 4) on the pressure-flow curves from dog perfused kidney. Symbols and vertical bars represent means and s.e., respectively. * shows a significant difference from the corresponding value of the control ($P < 0.05$).

increased level during the infusion of acetylcholine. At 10 min after the start of acetylcholine infusion at a perfusion pressure of 100 mmHg, the blood flow increased from 2·55 ± 0·42 to 3·18 ± 0·39 mL g^{-1} kidney weight min^{-1} (2 μg min^{-1}, $P < 0.05$) and to 3·40 ± 0·38 mL g^{-1} min^{-1} (5 μg min^{-1}, $P < 0.05$). The renal blood flow autoregulation during the infusion of acetylcholine was obviously present in a perfusion pressure range of 120 to 200 mmHg (Fig. 1A).

The intra-arterial infusion of carbachol (2 and 5 μg min^{-1}) at a perfusion pressure of 100 mmHg increased renal blood flow from 3·02 ± 0·36 to 3·41 ± 0·36 mL g^{-1} min^{-1} (2 μg min^{-1}, $P < 0.05$) and to 3·71 ± 0·39 mL g^{-1} min^{-1} (5 μg min^{-1}, $P < 0.05$) in a dose-dependent manner, and renal blood flow was maintained at an increased level during the infusion. The autoregulation was observed during the infusion of carbachol (Fig. 1B).

ARIs before and during the infusion of acetylcholine or carbachol are shown in Table 1. The control ARI values in all experiments were less than 0·3 between 120 and 200 mmHg, indicating an excellent autoregulation. The ARI value during infusion of acetylcholine or carbachol is not significantly different from the corresponding control value, it was also less than 0·3 between 120 and 200 mmHg, showing that the autoregulation was not influenced.

Discussion

The present data suggest that stimulating the muscarinic receptor does not influence renal blood flow autoregulation. This conclusion is based on the analysis of pressure-flow curve and the indexes of autoregulation during the infusion of acetylcholine and carbachol. We previously reported that prostaglandin E_2 and bradykinin had no effect on renal blood flow autoregulation in spite of the potent vasodilation (Ogawa & Ono 1985). Thus, it has been considered that not all vasodilators abolish renal autoregulation. Acetylcholine and carbachol are agents that do not influence autoregulation in spite of having vasodilator activity.

The previous study of Nahmod & Lanari (1964) had concluded that acetylcholine in doses higher than that used in the present study inhibited renal blood flow autoregulation. Although extensive comparison between our study and that finding does not seem warranted, the apparent difference may be related to the range of perfusion pressure observed. Nahmod & Lanari (1964) examined only the autoregulatory response below 140 mmHg of perfusion pressure, just under the autoregulatory range we found in the present

Table 1. Efficiency index of autoregulation (ARI) of renal blood flow.

	Renal perfusion pressure (mmHg)						
Drug	60–80	80–100	100–120	120–140	140–160	160–180	180–200
Control	1·60 ± 0·18	0·84 ± 0·04	0·39 ± 0·07	0·13 ± 0·05	0·09 ± 0·06	0·05 ± 0·06	0·14 ± 0·10
Acetylcholine							
2 μg min^{-1}	1·40 ± 0·10	0·95 ± 0·15	0·44 ± 0·09	0·15 ± 0·03	0·04 ± 0·02	0·01 ± 0·02	0·19 ± 0·11
5 μg min^{-1}	1·36 ± 0·19	0·70 ± 0·19	0·38 ± 0·12	0·16 ± 0·04	0·03 ± 0·02	0·04 ± 0·03	0·08 ± 0·02
Control	1·56 ± 0·22	1·06 ± 0·22	0·60 ± 0·21	0·24 ± 0·13	0·14 ± 0·05	0·12 ± 0·05	0·09 ± 0·05
Carbachol							
2 μg min^{-1}	1·27 ± 0·15	0·92 ± 0·15	0·55 ± 0·15	0·22 ± 0·08	0·14 ± 0·06	0·15 ± 0·06	0·17 ± 0·07
5 μg min^{-1}	1·17 ± 0·15	0·87 ± 0·17	0·57 ± 0·14	0·26 ± 0·08	0·20 ± 0·05	0·11 ± 0·05	0·09 ± 0·06

Values are means ± s.e. of each of 4 experiments.

study. Kiil et al (1969) also reported no effect of acetylcholine on renal blood flow autoregulation. However, they observed that the high dose infusion of acetylcholine abolished renal autoregulation. They explained this phenomenon by application of Poiseuille's law on the premise that acetylcholine acted mainly on muscle elements other than those participating in autoregulation.

We have observed that nitroglycerin, nicorandil, sodium nitroprusside and sodium nitrite, which are believed to relax smooth muscle through the activation of guanylate cyclase, had no effect on the autoregulation (Ogawa & Ono 1986). Furchgott & Zawadzki (1980), and Murakami et al (1985) reported that acetylcholine- and carbachol-induced relaxation of vascular smooth muscle was due to the endothelium-derived relaxing factor (EDRF) which is released from endothelial cells. Furthermore, some investigators have shown that EDRF produces an increase in cGMP by stimulation of guanylate cyclase in vascular smooth muscle (Furchgott et al 1981; Rapoport & Murad 1983; Ignarro et al 1984). If renal vasodilation induced by the infusion of acetylcholine and carbachol into the renal artery is mediated by increase of cGMP, the present results are supported by our previous data showing that vasodilators that relax smooth muscle through cGMP did not abolish renal autoregulation.

Ono et al (1974) showed that the intrarenal infusion of verapamil (30 $\mu g\ min^{-1}$) or nifedipine (3 $\mu g\ min^{-1}$) abolished renal autoregulation in spite of not showing vasodilating action at low perfusion pressure. Thus, there does not seem to be a direct correlation between the vasodilating mechanism and the autoregulatory mechanism. In our present experiment, the lack of influence of acetylcholine and carbachol on the autoregulation cannot be accounted for by insufficiency of the doses used. Due to the negative finding of our study, we concluded that muscarinic receptors do not contribute to the mechanism of autoregulation of renal blood flow.

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Evaluation of sorghum starch as a tablet disintegrant and binder

A. V. Deshpande*, L. B. Panya, *Dept of Pharmaceutics & Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria*

The starch prepared from the seeds of *Sorghum bicolor*, Moench has been evaluated as a disintegrant and binder in tablets of magnesium sulphate, calcium carbonate, sulphadimidine, and chloroquine phosphate to represent soluble and insoluble inorganic and organic substances. The starch performed as well as maize starch in binding and disintegrating properties and better than acacia as binder.

* Correspondence.

While starch from different plants is an adjuvant in the formulation and production of solid dosage forms, starch from sorghum, a food crop widely grown in tropical Africa, including Nigeria, appears not to have been used as a tablet excipient. We have examined its usefulness both as a binder and as a disintegrant for the formulation of tablets containing inorganic and organic medicinal substances and compared the physical characteristics of the tablets with those prepared using acacia as a binder and maize starch as binder/disintegrant. Magnesium sulphate and calcium carbonate were used as soluble and insoluble inorganic substances, and sulphadimidine and chloroquine phosphate as insoluble and soluble organic substances, respectively.

Materials and methods

Sulphadimidine (May & Baker Ltd, UK) and chloroquine phosphate (Bayer Nigeria Ltd) were of BP grade. Maize starch and acacia were from BDH Chemicals Ltd and May & Baker Ltd UK, respectively.

Sorghum starch was prepared in our laboratory from the seeds of sorghum (*Sorghum bicolor*, Moench)