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Carbachol, an acetylcholine receptor agonist, enhances production in rat aorta of 2-arachidonoyl glycerol, a hypotensive endocannabinoid

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Abstract

The production of 2-arachidonoyl glycerol, an endogenous cannabinoid, is enhanced in normal, but not in endothelium-denuded rat aorta on stimulation with carbachol, an acetylcholine receptor agonist. 2-Arachidonoyl glycerol potently reduces blood pressure in rats and may represent an endothelium-derived hypotensive factor.

Keywords: Hypotension; 2-Arachidonoyl glycerol; Cannabinoid

Stimuli that dilate blood vessels, such as acetylcholine or carbachol, lose their vasodilating activity if the blood vessels are stripped of endothelium. This tissue releases an endothelium-derived relaxing factor (EDRF) when activated by the above stimuli. The endothelium-derived relaxing factor has been identified as nitric oxide (NO), which is a physiological mediator of blood vessel relaxation, and hence plays a major role in the regulation of tissue blood flow (Moncada et al., 1991). Additional vasodilating factors such as prostacyclin are also released from the endothelium of various tissues. The existence of a factor, tentatively named endothelium-derived-hyperpolarizing factor (EDHF), has been postulated. Anandamide (arachidonoyl ethanolamide) an endogenous cannabinoid (Devane et al., 1992) has been suggested as a likely candidate (Randall and Kendall, 1998). However this suggestion has not been generally accepted (Edwards and Weston, 1998).

We report now that, in isolated rat aorta under cholinergic stimulation by carbachol, the production of 2-arachidonoyl glycerol, a second type of endogenous cannabinoid (Mechoulam et al., 1995) is significantly enhanced. Varga et al. (1998) have recently shown that lipopolysaccharide-treated macrophages and platelets produce anandamide and 2-arachidonoyl glycerol, respectively, both of which cause hypotension when administered to rats. The hypotension elicited by lipopolysaccharide-treated macrophages or platelets remained unchanged after blockade of nitric oxide synthetase, but was inhibited by the cannabinoid CB_1 receptor antagonist SR141716A (Varga et al., 1998). The hypotension produced by 2-arachidonoyl glycerol has now been independently confirmed. Together these observations point toward the possibility that 2-arachidonoyl glycerol is an endogenous physiological mediator of blood vessel relaxation.

Male rats (Sabra strain, 270–350 g) were killed by cervical fracture. Internal thoracic and abdominal organs were quickly removed exposing the aorta. The aortic arch was cannulated, renal and mesenteric arteries were clamped, and the lower end of the aorta was cut at the iliac bifurcation to enable free perfusion of Krebs–Henseleit solution through the aorta. The solution was gassed with a mixture of 95% 0_2 and 5% CO_2 to achieve a pH of 7.4 and was perfused by gravity at 10 ml/min for 10 min at room temperature. Cholinergic stimulation was applied by addition of carbamyl-choline chloride (carbachol) (10^{-5} M, Sigma) into the solution. Endothelium denuded aortic preparations were made by perfusion of the aorta with distilled water for 10 min. Following perfusion, the aorta was removed, weighed and kept at −70°C for analysis.

Rat aorta tissues (~0.5 g, from 5 rats) were homogenized in chloroform/methanol (2:1 v/v), and 5 nmol

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2-eicosanoyl glycerol, as internal standard, was added during the homogenization. The homogenate was centrifuged at $3800 \times g$ for 20 min. The extract was worked up as previously described and was purified by thin-layer chromatography (Mechoulam et al., 1995). Each experiment was performed twice. The band that had the same Rf as synthetic 2-arachidonoyl glycerol was scraped off and extracted with chloroform:methanol (85:15, v/v), dried under nitrogen and stored at −20°C. Quantitative gas chromatography mass spectrometry analysis was performed as described in Ben-Shabat et al. (1998). The data were analyzed with non-parametric (Mann–Whitney) tests.

Systemic blood pressure and rectal temperature were monitored in male rats (Sabra strain, weighing 270–350 g). As 2-arachidonoyl glycerol, an ester, is rapidly hydrolysed in vivo, we tested the effects on blood pressure of both 2-arachidonoyl glycerol and of a stable analog, 2-arachidonyl glyceryl ether (HU-310), in which the labile ester moiety of 2-arachidonoyl glycerol is replaced by a stable ether one. Blood pressure was measured via a chronic cannula (PE 50, Clay Adams) implanted into the femoral artery under sodium pentobarbital anesthesia (60 mg/kg) (Shochina and Horowitz, 1989). On the experimental day, the animal was lightly restrained and the cannula was attached to a pressure transducer (Db23, Statham). The transducer was connected to a data acquisition system (CODAS software and scroller, Dataq) and the pressure was sampled at a rate of 1/s and stored on a computer disk. Rectal temperature was monitored with a thermistor probe (402 probe, Yellow Springs Instruments, OH). Both recordings were taken for 30–60 min before treatment and for 75 min following i.v. bolus injection of 2-arachidonoyl glycerol or of 2-arachidonyl glyceryl ether. Each rat received either drug at several doses (4, 8 or 12 mg/kg). Each successive dose was administered always after the blood pressure had returned to its baseline value. Dose-related responses were observed (data not shown). Time curves for 12 mg/kg of each drug were compared by two-way analysis of variance (ANOVA). Changes from baseline values for each drug were compared by one-way ANOVAs, followed by post-hoc Newman–Keuls tests.

The levels of 2-arachidonoyl glycerol in aortas after carbachol treatment were compared with those in aortas that had not undergone such a treatment (two experiments; $n = 5$ in each experiment). The level of 2-arachidonoyl glycerol in the controls was 0.41 nmol/g wet weight in the first experiment and 0.70 nmol/g in the second. In the carbachol-treated aortas the values were 5-fold higher: 2.0 nmol/g in the first experiment and 3.78 nmol/g in the second ($P < 0.001$). The levels of anandamide in both the controls and the carbachol treated aortas were below the sensitivity of our gas chromatography mass spectrometry instrument (i.e., below 50 pmol/g). In aortas which had been denuded from endothelium, the level of 2-arachidonoyl glycerol was 0.5 nmol/g and it did not increase on treatment with carbachol.

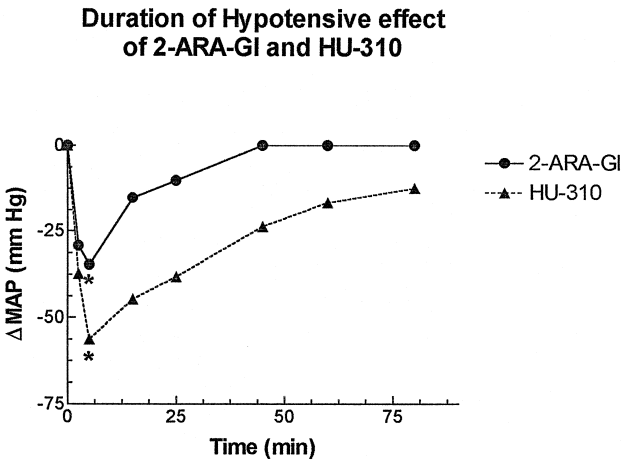

Fig. 1. Duration of hypotensive effect (ΔMAP = change in mean arterial pressure) of 2-arachidonoyl glycerol (2-Ara-Gl) and 2-arachidonyl glyceryl ether (HU-310). * $P < 0.05$ for difference between 2-Ara-Gl, HU-310 and their respective baseline values.

The baseline mean arterial pressure values were 122 ± 14 and 120 ± 4 mm Hg for the 2-arachidonoyl glycerol and for the 2-arachidonyl glyceryl ether assays, respectively. 2-Arachidonoyl glycerol (12 mg/kg) caused a short-lived decrease in mean arterial pressure on i.v. administration of 40 ± 10 mm Hg which waned after 18 min. At this dose the rectal temperature of the rat was reduced by 2.3 ± 0.26°C. The synthetic 2-arachidonyl glyceryl ether (HU-310) reduced blood pressure more strongly, by 66 ± 12 mm Hg, an effect which lasted for more than 40 min. These data are presented in Fig. 1. The curve from the 2-arachidonoyl glycerol treated animals differed significantly from the HU-310-induced curve ($P < 0.002$).

In conclusion, the above data show that stimulation of rat aorta with carbachol enhances the synthesis of 2-arachidonoyl glycerol by this tissue. This endogenous cannabinoid causes reduction of blood pressure. Further work, in particular determination of membrane polarization and hyperpolarization of the aorta in rats, is needed to establish whether 2-arachidonoyl glycerol is a putative endothelium-derived hyperpolarizing factor.

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