the increase in ^{14}C in the TCA fraction is not due to its incorporation into amino acids and thus much of this increased radioactivity may occur as glucose or glycolytic metabolites. GEY, RUTISHAUSER and PLETSCHER[11] have described a rise in brain glucose in CPZ hypothermia in rats, which they attributed to suppression of glycolysis, but prevention of hypothermia did not abolish changes in carbohydrate metabolism produced by CPZ[12].

Zusammenfassung. Es wird gezeigt, dass Chlorpromazin keine Veränderung der Konzentration von Aminosäuren erzeugt, wohl aber die Inkorporation des Isotopen ^{14}C-Glukose im säurelöslichen Anteil des Mäusegehirns temperaturabhängig vermehrt.

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The Relationship of the Renal Vasodilator Action of Bradykinin to the Release of a Prostaglandin E-Like Substance

Kinins have variable effects on blood vessels; viz., in vitro constriction usually occurs, whereas in vivo most vascular elements dilate, although venoconstriction is the rule[1]. The diverse vascular effects of kinins are evident in the actions of bradykinin on the fetal circulation. Bradykinin constricts the umbilical vessels and the ductus arteriosus, and dilates the pulmonary vasculature which effects have occasioned the proposal that a kinin mediates neonatal circulatory changes[2]. If some of the effects of kinins on blood vessels can be shown to be dependent on release of a mediator, then their diverse vascular actions may be made comprehensible. Postaglandins of the E (PGE) and A (PGA) series have been suggested to be local mediators of stimuli evoking vasodilation[3]. We undertook the present study in an attempt to relate the renal vasodilator action of bradykinin to the release of prostaglandins. The renal circulation was selected since renal blood flow (RBF) is highly sensitive to prostaglandins and kinins[4,5] and the predominent renal prostaglandin, PGE_2[6,7] is released by vasoactive hormones[8].

Methods. Male mongrel dogs (22–31 kg) were anesthetized with morphine sulfate (2 mg/kg, s. c.) and chloralose (100 mg/kg, i.v.). The abdominal cavity was opened by a transverse incision and a renal artery isolated. Two Hewlett-Packard direct writers recorded: a) mean aortic blood pressure measured by a Statham transducer; b) RBF measured by a Statham electromagnetic flowmeter and c) changes in length of assay organs detected by Harvard isotonic transducers. We have reported the adaptation of the blood-bathed organ technique of VANE[9] for continous assay of prostaglandins in renal venous effluent[10] (Figure). In brief, 3 assays organs: rat stomach strip, rat colon and chick rectum, were superfused (streaming of fluid over assay organs) in series by renal venous blood withdrawn by a pump at 15 ml/min and returned to the dog via the left jugular vein. The assay organs in vitro were superfused with Krebs solution in order to estimate concentrations of PGE- and PGF-like substances in purified extracts of renal venous blood. Renal venous blood (100 ml) was collected in ethanol before and during infusion of bradykinin into the renal artery. Heparin (1500 IU/kg) was given i.v. just prior to superfusing the assay tissues. Dextran was infused i.v. at the same rate as renal venous blood was removed. The ethanolic-blood mixture was filtered, evaporated and the acidic lipids separated from the neutral lipids as previously described[11]. The acidic lipids were further purified by thin-layer chromatography on silica gel layers, 0.5 mm thick, using the solvent system: chloroform: methanol: acetic acid (18:1:1 by vol.). Eluates from thin-layer chromatographic zones were reconstituted in Krebs solution to make a final dilution of 0.5 ml; 0.1 ml volumes of the latter were assayed in vitro for prostaglandins. Since the minimum amount of PGE_2 standard which produced a measurable response of the assay organs varied between 0.1 and 0.3 ng, the threshold of sensitivity of this assay system for PGE-like substances expressed as PGE_2 equivalents was always 0.015 ng/ml blood or less. Thus, the sensitivity of this assay for PGE_2 is well below the threshold value of PGE_2 of 0.1 ng/ml blood which increases RBF[4]. The concentration of PGE- and PGF-like substances in the eluate was determined by bracket assay (Figure). The medians of the coefficients of variation of the assay system were 10.9 and 12.9% respectively for duplicate and replicate determinations of the concentration of prostaglandins in the eluates. Concentrations of prostaglandins were not corrected for losses (average 38%) incurred on extraction and purification.

Results. Bradykinin, given into the renal artery by infusion (20 to 100 ng/kg/min) or single shot 40 ng/kg, increased RBF by 16 to 110% of control (mean increase 58%). Aortic blood pressure was unchanged from the mean control value of 96 mm Hg. In all experiments, contraction of the assay organs bathed by renal venous blood occurred in response to close-arterial administration of bradykinin (Figure). Bradykinin presumably released a

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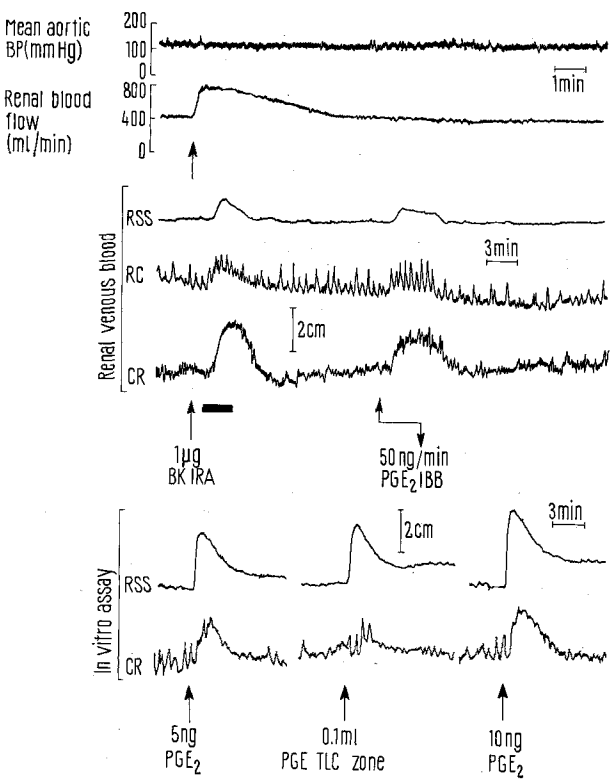
Effect of bradykinin on renal blood flow, blood pressure and concentrations of PGE- and PGF-like substances in renal venous blood

	Renal blood flow (ml/min)[a]	Mean aortic blood pressure (mm Hg)[a]	Prostaglandin (ng/ml blood)	
			E-like (E_2 equivalents)[a]	F-like ($F_2\alpha$ equivalents)[a]
Control	282±40	96±7	0.27±0.11	0.27±0.16
Bradykinin	444±61	94±7	1.35±0.24	0.45±0.20
Probability levels[b]	<0.01	NS[c]	<0.01	NS[c]

[a] Values are mean±S.E.M. for 10 experiments in 10 dogs. Bradykinin was infused into the renal artery at 20–100 ng/kg/min (8 experiments) or was given by single shot, 40 ng/kg (2 experiments). [b] Statistical analyses were made, using the paired *t*-test, for experimental (bradykinin) versus control periods. [c] NS indicates no statistical significance.

prostaglandin(s), since PGE_2 standard, but not bradykinin, infused directly into the extracorporeal circuit, thereby bypassing the kidney, reproduced the effects of intra-arterial administration of bradykinin on the assay organs. The effects of bradykinin on RBF, blood pressure and concentrations of PGE- and PGF-like substances in renal venous blood determined by in vitro assay are shown in the Table. Bradykinin increased the concentration of a PGE-like substance in renal venous blood 5-fold: range 0.25 to 2.17 ng/ml. Bradykinin produced variable and insignificant changes in the concentration of a PGF-like substance (Table).



Effects of bradykinin (BK) injected into the renal artery (IRA) on mean aortic blood pressure (BP), renal blood flow and assay organs superfused by venous blood of the same kidney in a chloralose-anesthetized dog. PGE_2 standard infused into the extracorporeal circuit (IBB) matched the contractions of the assay organs produced by infusion of bradykinin IRA. The time of collection of renal venous blood is indicated by the black bar. PGE zones of chromatographed extracts of this blood produced contractions of the assay organs superfused by Krebs solution similar to PGE_2 standards (lowest panel: in vitro assay). Thus, the renal vasodilator action of bradykinin occurred simultaneously with the appearance in renal venous blood of a substance having the properties of PGE_2. RSS, rat stomach strip; RC, rat colon; CR, chick rectum; TLC, thin-layer chromatography.

Discussion. The variable action of kinins on blood vessels[1] may be the consequence of direct and indirect actions, the latter determined by the release of a prostaglandin. There is compelling evidence that the action of bradykinin on effector organs depends partially on indirect effects resulting from release of mediators[12]. This proposal has been considerably strengthened by the recent demonstration that aspirin, which blunts some of the vascular actions of kinins[12], inhibits the synthesis of prostaglandins[13]. The present study, although suggestive of an indirect action of bradykinin on RBF, cannot exclude a complementary or even antagonistic direct effect of bradykinin. Final identification of PGE_2 as the mediator of the bradykinin-induced renal vasodilation awaits definitive chemical verification which was precluded in the present study by the extremely small concentration of a PGE-like substance in renal venous blood. However, several observations suggest that this material is PGE_2; viz., a) PGE_2 is the predominant renal prostaglandin[6,7]; b) the substance tentatively identified as PGE_2 in renal effluent has been characterized by argentous thin-layer chromatography as the more unsaturated prostaglandin of the E series[10], PGE_2; and c) PGE_2 when infused intra-arterially at a rate which produced concentrations in renal blood comparable to those of a PGE-like substance evoked by bradykinin, increased RBF similarly to the kinin[4]. The selective release by bradykinin of PGE_2, a vasodilator, and its failure to release $PGF_2\alpha$ which constricts some vascular elements[14] provide additional evidence albeit inferential, that the release of PGE_2 by bradykinin is substantive to its vasodilator action. We cannot comment on the participation of the third renal prostaglandin, PGA_2, in the renal vasodilator action of bradykinin, since PGA_2 having negligible smooth muscle stimulating activity cannot be measured by the assay method. However, PGA_2 has only $^1/_5$ the renal vasodilator potency of PGE_2 and may be largely artifactual[6]. Some of the objections to assigning physiological or pathological roles to kinins[15] such as functional vaso-

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ditation[16] or carcinoid flush[17], respectively, may be answered if the activity of kinins on blood vessels depends on release of a mediator(s) having similar or even antagonistic actions to kinins[18].

Zusammenfassung. Nachweis, dass Bradykinin den renalen Blutstrom um 58% der Kontrollwerte (282 ± 40 ml/min) erhöht und gleichzeitig im venösen Nierenblut die Konzentration einer Substanz, welche die physicochemischen und biologischen Eigenschaften eines Prostaglandins der E-Serie besitzt, steigert, wobei die Konzentration einer PGF-ähnlichen Substanz unverändert blieb.

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Effect of 5-Hydroxydopamine on Uptake and Content of Serotonin in Rat Striatum

The synthetic phenylethylamine derivative 5-OH-dopamine is able to act as a false transmitter, i.e. it can replace norepinephrine in the storage vesicles[1,2] and is released from sympathetic nerves[1]. The advantage of 5-OH-dopamine in animal experiments is its intense osmiophilic property, so that the replacement of norepinephrine can be observed with the help of the electron microscope. Like L-dopa, the analogue 5-OH-dopa is also decarboxylated to the respective amine, i.e. 5-OH-dopamine. It is known that treatment of several species with L-dopa causes a decrease in the serotonin (5-HT) content of brain[3–6], which might be due to enhanced release[3,7] and metabolism[3] of 5-HT. Therefore, it was of interest to know whether 5-OH-dopa acted similarly to L-dopa. The serotonin content of the striatum was measured in rats after treatment with 5-OH-dopa. Further, the influence of 5-OH-dopamine on the uptake of tritiated serotonin by striatal tissue slices of rats was studied in vitro.

Materials and methods. 1. In the in vivo experiments (male Sprague-Dawley rats, 120–150 g) 5-OH-dopa was injected twice in a dose of 400 mg/kg i.p. 15 and 4 h before decapitation. The serotonin content of rat striatum was determined according to the method of Snyder et al.[8].

2. In vitro, striatal tissue slices of rats, prepared according to McIlwain et al.[9], were incubated at 37 °C in a glucose Ringer's solution with addition of 0.2 mg/ml ascorbic acid and under constant gas supply (95% O_2, 5% CO_2). After 10 min of pre-incubation ^{3}H-serotonin (spec. activity 8.5 C/mM, Radiochemical Center, Amersham) was added to the incubation medium to obtain a final concentration of $5.9 \times 10^{-7} M$. After 30 min of incubation the slices were rinsed in fresh buffer and blotted with filter paper. The slices were weighed and solubilized in 0.5 ml Soluene TM_{100} (Packard Instr. Co. Inc.). Radioactivity of each tissue slice was counted in a liquid scintillation spectrometer (LS-200B, Beckman). The uptake of ^{3}H-5-HT was determined by calculating the tissue to medium ratio of the isotope after correction by the external standard ratio method. 5-OH-dopamine was added after the preincubation period in concentrations of 0.001–1.0 mg/ml.

3. For electron microscope preparation, the incubated tissue slices (see above) were rapidly fixed in glutaraldelhyde fixative with phosphate buffer (pH 7.4) for 1 h. They were then washed in phosphate buffer and post-fixed in

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Uptake of ^{3}H-serotonin ($5.9 \times 10^{-7} M$) by striatal tissue slices of rats in vitro under the influence of 5-OH-dopamine

	Control	5-OH-dopamine (μg/ml)			
		1000	500	10	1
Uptake ratio $\bar{x}$ ± S.E.M.	11.85 ± 0.87	3.75 ± 0.15	4.06 ± 0.26	6.86 ± 0.47	9.37 ± 0.37
No. of experiments	15	10	13	12	8
% of control	100	32	34	58	79
P		< 0.001	< 0.001	< 0.001	< 0.02

For details, see methods. p was calculated according to the Student's t-test.