It is likely that the inhibitor was removed by a photooxidative reaction. Sweetser also illuminated a solution of flavin mononucleotide and CMU for several hours and was able to isolate a reaction product of high molecular weight; we consider this to be the result of an irreversible photooxidative destruction of an initial CMU-flavin complex, the formation of which may be highly favored by the similarity in structure between CMU and flavin (see *8*). An association between these molecules might be responsible for the effects we observed. For example, such an association should facilitate a "chemical quenching" process, as has been discussed by Matsumoto and by Eigenmann for other systems (*9*).

The significance of our results with CMU in relation to photosynthesis is not immediately apparent, since we observed changes in the reactivity of light-excited flavin molecules, and so far, these are not believed to be part of the photosynthetic mechanism. Yet the properties of chloroplast flavin may be changed by its forming a complex with CMU so that it diverts the precursors to free oxygen from their regular pathway (*10*).

PETER HOMANN
HANS GAFFRON

Florida State University, Institute of Molecular Biophysics (Fels Fund), Tallahassee, Florida

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Bradykinin: Vascular Relaxant, Cardiac Stimulant

Abstract. *Bradykinin infusion causes an increase in cardiac output in rats whether the autonomic nervous system is blocked or not. After autonomic blockade, bradykinin causes a lesser decrease in the total peripheral resistance but a greater increase in cardiac output, resulting in an elevation of arterial pressure. The increase in cardiac output is caused by a small increase in heart rate and a substantial increase in stroke volume. The fact that these increases are observed after autonomic blockade suggests that bradykinin increases cardiac output by direct stimulation of the heart.*

Bradykinin is known primarily as a potent relaxant of vascular smooth muscle, having a hypotensive effect. The results of our study, however, indicate that this effect is greatly modified or even reversed by its direct stimulating action on the heart. It is known that bradykinin causes an increase in cardiac output in man (*1*) and in the intact dog (*2*). This might be caused by (i) a secondary neurogenic compensation for the hypotensive effect, or (ii) by a direct positive inotropic action or a direct positive chronotropic action, or both, on the heart. The second possibility is in accordance with the observation that the polypeptide causes an elevation in arterial pressure when administered to rats that have had their autonomic nervous systems blocked (*3*). However, this pressor response could be caused either by an increase in cardiac output or by a reversal of the usual vasodilator effect of bradykinin. The current study was undertaken to evaluate the possibility that the pressor effect of bradykinin is evidence of its direct stimulating action on the heart.

The mean arterial pressure (MAP), cardiac output (CO), and heart rate (HR) were measured during infusion of synthetic bradykinin (*4*) into rats before and after ganglionic blockade. From these values, the total peripheral resistance (TPR) and stroke volume (SV) were calculated. Ten male rats weighing 195 to 280 g were used. They were anesthetized with sodium pentobarbital (40 mg/kg by intraperitoneal injection) and heparinized (10 mg/kg by intravenous injection). Figure 1 shows the details of the technique. The MAP was recorded directly from the femoral artery; the CO was measured by a thermodilution technique (*4, 5*). The indicator (0.1 ml saline at room temperature) was injected into the central venous pool through a cannula in the right external jugular vein. The resultant thermodilution curve was monitored from a thermistor placed in the ascending aorta via the right carotid artery. The CO measurements were made at 2-minute intervals during the control and infusion periods. Bradykinin was infused into the right femoral vein at 12.5 (slow rate) and 25.0 (fast rate) μg/kg per minute. The duration of infusion was 5 minutes and another period of 5 minutes was allowed after each infusion for the destruction of bradykinin. The autonomic nervous system was blocked by pentolinium bitartrate (5 mg/kg by intraperitoneal injection). In each experiment, responses to the two infusion rates of bradykinin were measured before and after pentolinium was administered.

Results of one experiment are given in Fig. 2 and the average of the results of ten experiments are given in Table 1. Without ganglionic blockade, infusion of bradykinin caused an initial decrease in MAP; this decrease was transient, the pressure always returning toward control levels early in the infusion period. The CO increased, reflecting a slight increase in HR and a substantial increase in SV. The TPR fell precipi-

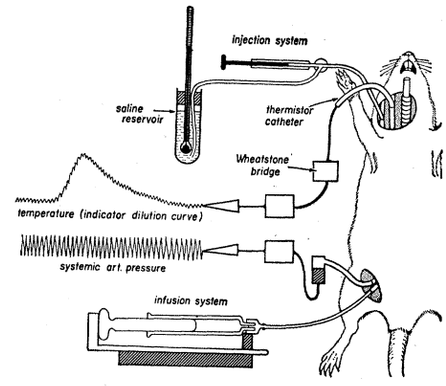

Fig. 1. Thermodilution technique for measurement of cardiac output. The closed system for injection of the indicator is at room temperature (20-25°C). Saline is withdrawn from the reservoir where its temperature is known to the nearest 0.1°C, into a Hamilton microliter syringe; it is then injected rapidly into the rat. Dilution of the indicator (change in temperature) is recorded by a thermistor (Fenwall Electronics, GC32J1) which has a time constant in saline of 0.12 second. Changes are recorded on a Grass model 5 polygraph.

Table 1. Hemodynamic changes caused by infusion of bradykinin.

Infusion rate (μg/kg/min)	MAP (mm-Hg)	CO (ml/kg per min)	HR (beats/min)	SV (ml/kg)	TPR (PRU*/kg)
		Rat intact			
12.5	-25 ± 8.4†	$+ 82.6 \pm 18.4$	$+ 6 \pm 11$	$+0.19 \pm 0.02$	-0.135 ± 0.026
25	-42 ± 4.4	$+ 89.4 \pm 17.8$	$+14 \pm 14$	$+0.17 \pm 0.03$	-0.194 ± 0.024
		Rat after ganglionic blockade			
12.5	$+14 \pm 0.25$	$+ 93.5 \pm 14.9$	$+21 \pm 5.1$	$+0.21 \pm 0.04$	-0.028 ± 0.008
25	$+18 \pm 2.4$	$+148 \pm 13.8$	$+53 \pm 8.2$	$+0.29 \pm 0.04$	-0.058 ± 0.012

* Unit of peripheral resistance. † $\pm$ Indicates standard error of the mean.

tously with the onset of infusion, but often recovered somewhat as infusion continued. Lack of a dose-dependent relationship of the depressor effect of bradykinin was frequent (see MAP, Fig. 2). Such a complex relationship is not surprising when an agent has opposite effects on two determinants (TPR and CO) of an observed response (MAP). Nearly all the changes produced by bradykinin in animals without ganglionic blockade differed in relation to control levels with P values $< .005$. Exceptions were the depressor effect at the slow infusion rate ($.01 > P > .005$) and the change in heart rate at either infusion rate ($P > .25$).

In rats with ganglionic blockade, bradykinin caused an increase in MAP; the pressure remained elevated throughout the infusion period. The CO increased as it did before blockade. This increase was caused by an increase in both HR and SV. After blockade, however, there was an impressive dependence of the increase in CO on the dose, which seems to have been obscured when the autonomic nervous system was intact (Fig. 2 and Table 1). The TPR decreased but the magnitude of the decrease was not as great as before blockade. All changes, produced by bradykinin after ganglionic blockade, differed in relation to post-blockade control levels, with P values $< .005$.

When the effects of bradykinin after blockade are compared with those produced before, the differences in both MAP and TPR changes are highly significant ($P < .005$). Lesser levels of significance are found when this comparison is made of the bradykinin effect on HR (slow rate of infusion, $.025 > P > .01$; fast rate, $.05 > P > .025$) and CO (fast rate, $.025 > P > .01$). In other parameters, the change produced by bradykinin after blockade did not differ significantly from those before.

These observations constitute strong evidence that bradykinin has a potent, direct stimulating action on the heart, since the alternative mechanisms furnish less probable explanations for the observed increase in CO.

1) The increase in CO could be due to the direct effect of a fall in arterial pressure. However, such a mechanism could not account for the observed increase in CO after autonomic blockade, when the arterial pressure is simultaneously increased, or for the persistent increase in CO in the animal without blockade when MAP returns toward control values during the latter part of the 5-minute infusion period.

2) The increase in CO could be due to an increase in sympathetic outflow to the heart which would be expected to result from the decrease in baroreceptor activity during hypotension. However, this mechanism could not account for the observed increase in CO after the sympathetic outflow to the heart has been eliminated by autonomic blockade.

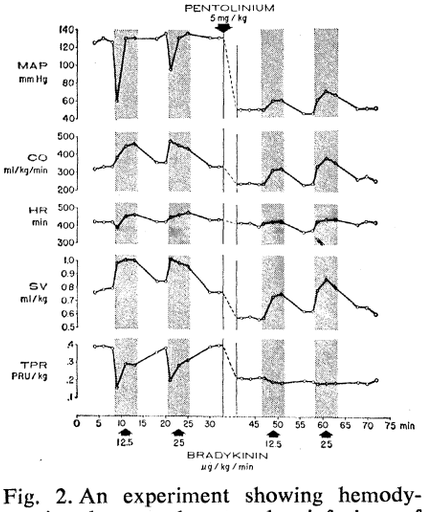

Fig. 2. An experiment showing hemodynamic changes due to the infusion of bradykinin: MAP, mean arterial pressure; CO, cardiac output; HR, heart rate; SV, stroke volume; TPR, total peripheral resistance. In each crosshatched column the first recorded observations were made one minute after the beginning of infusion.

3) It is possible that the increase in CO in response to bradykinin infusion could be caused by an increase in venous return, with a resultant increase in ventricular filling pressure. Although the current study affords no direct evidence against this possibility, it seems improbable that a potent relaxer of vascular smooth muscle would cause an increase in venous return.

4) Since bradykinin has been shown to be a potent coronary vasodilator (*6*), the possibility cannot be ruled out that the enhanced CO is due to an improved myocardial blood flow. This seems unlikely, since the rat heart, in the Langendorff preparation (*6*), shows this dilator effect only poorly.

These arguments by exclusion suggest, but do not prove, that bradykinin has a direct cardiac-stimulating action in vivo. This possibility is supported by the report that bradykinin has positive chronotropic and positive inotropic actions in the isolated heart (*6*).

The dual action of bradykinin, which is evident from the current experiments, is a striking example of the fact that measurement of arterial pressure may mask important details of a hemodynamic response. Late during the infusion period in rats without ganglionic blockade, the arterial pressure was often at control levels while the TPR was greatly decreased and CO equivalently increased. The cardiac-stimulating action of bradykinin, regardless of the mechanism responsible, is a major part of its hemodynamic effect in the rat (*7*).

DROGO MONTAGUE
RAMON ROSAS*
DAVID F. BOHR
Department of Physiology, University of Michigan, Ann Arbor

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* Present address; Laboratorio de Fisiología, Escuela de Medicina, Universidad Católica de Chile, Santiago, Chile.

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