J. Pharm. Pharmacol. 1988, 40: 721–723
Communicated April 15, 1988

Cardiovascular effects of intracerebro-ventricular bradykinin and melittin in the rat

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Abstract—The cardiovascular effects of intracerebroventricular (i.c.v.) bradykinin and melittin were investigated in the anaesthetized rat. Bradykinin, 30 μg, increased mean arterial pressure by 15 mmHg and this was the result of an increase in peripheral resistance; heart rate and cardiac output were unchanged. Tissue blood flow was lower in the skin and spleen in the animals given bradykinin than the controls. Significant increases in tissue vascular resistance occurred in the skin and several organs of the splanchnic region, the spleen, stomach, large intestine and the pancreas/mesentery. Melittin infusion gave a biphasic response in systemic blood pressure in which a depressor response was followed by a pressor phase; the pressor stage was accompanied by an increase in heart rate. Since melittin is a stimulant of membrane bound kallikrein, the results lend limited support to the hypothesis that there is a kallikrein-kinin system endogenous to the central nervous system which is involved in cardiovascular regulation.

There is an increasing amount of evidence for the presence of a functional kallikrein-kinin system in the central nervous system. Thus, the presence of a kininogen substrate was suggested by Shikimi et al (1973) who showed that incubation of extracts of rat brain tissue with trypsin produced kinin-like material. More recently, Chao et al (1984) demonstrated a low molecular weight kininogen present in extracts of rat brain. Also in rat brain is the enzyme which converts the kininogen to kinin; an antibody to rat urinary kallikrein has been used to purify kallikrein from the brain and the enzyme has been produced in a cell-free system using rat brain mRNA (Chao et al 1983). Finally, Perry & Snyder (1984) demonstrated the presence of authentic bradykinin within the rat central nervous system and this could be the product of brain kallikrein acting on endogenous kininogen.

Such a brain kallikrein-kinin system may have a role in cardiovascular regulation since administration of bradykinin into the cerebral ventricles of the rat increases systemic blood pressure (Pearson et al 1969; Corrêa & Graeff 1974, 1975). Local injections of lower doses of bradykinin into the lateral septal area also give pressor responses and the effect of intracerebroventricular (i.c.v.) kinin is abolished by lesions in this region (Corrêa & Graeff 1975). This response might be linked to central neurons releasing bradykinin since Corrêa et al (1979) showed that fibres containing bradykinin-like immunoreactive material were present in this region although only the hypothalamus possessed cell bodies with bradykinin-like material. The possible involvement of the hypothalamus as a site for bradykinin-mediated cardiovascular control is further indicated by observations that injections of bradykinin into discrete nuclei of the dorsomedial, posterior and anterior hypothalamus can increase both blood pressure and heart rate (Diz & Jacobowitz 1984) and that tissue kallikrein is present in hypothalamic cell bodies (Simson et al 1985).

That the central kallikrein-kinin system is functional is suggested by observations with melittin, a component of bee venom (Habermann 1972), which can activate membrane-bound kallikrein (Nishimura et al 1980). In the dog introduction of this basic polypeptide into the cerebral ventricular system increased both levels of cerebrospinal fluid kinin-like immunoreactive material and mean arterial pressure (Thomas et al 1984). Other support comes from experiments in which stimulation of the afferent fibres of the vagi was shown to produce simultaneous increases in mean arterial blood pressures and cerebrospinal fluid kinins (Thomas et al 1987).

Systemic pressor responses can be produced by changes in cardiac output, peripheral resistance or both and the contributions made by these two parameters to changes in blood pressure may be readily measured by tracer microspheres (McDevitt & Nies 1976). Here we report an investigation intended to determine the vascular regions involved in the pressor responses in rats brought about by administration of bradykinin into the cerebral ventricles. We have also attempted to activate an endogenous kallikrein-kinin system in the rat central nervous system by intracerebroventricular (i.c.v.) infusion of mellitin. A preliminary report of this study was presented to the British Pharmacological Society meeting in London, December 1986 (Hiley & Thomas 1987).

Methods

Determination of cardiac output and tissue blood flow after i.c.v. bradykinin. Male Wistar rats (Bantin & Kingman, Hull) were anaesthetized with 120 mg kg^{-1} sodium thiobutabarbitone (Inactin; BYK Gulden, Konstanz, FRG) and both femoral arteries were cannulated; one to allow withdrawal of blood and the other for continuous recording of blood pressure by a Bell & Howell pressure transducer (Type 4-422-0001) connected to a Grass Model 79D polygraph. Heart rate was derived from the pressure wave with a Grass Model 7P4 tachograph. With the aid of pressure monitoring a third cannula was passed down the right common carotid artery and into the left cardiac ventricle.

After these cannulations, the animals were placed in a stereotaxic frame and a micro-drill was used to make a hole in the skull 1·5 mm lateral and 1·5 mm caudal to the bregma on the left side. A stainless steel cannula of 0·45 mm diameter was then inserted to a depth of 3·5 mm and thus into the left lateral cerebral ventricle (Noble et al 1967). 30 μg bradykinin (Sigma) was injected over 10 s through this cannula in a volume of 30 μL artificial cerebrospinal fluid of the following composition (Merlis, 1940): NaCl 138·5, KCl 3·35, $CaCl_2$ 1·26, $MgCl_2$ 1·16 $NaHCO_3$ 21 NaH_2PO_4 0·5, urea 2·2, glucose 3·39 mM. Control animals received 30 μL of the vehicle only.

A stable response to bradykinin occurred within 2 min and so cardiac output and its distribution were determined at that time in the experimental and the control animals. 60,000–80,000 plastic microspheres, of 15±3 μm diameter and labelled with ^{113}Sn, were suspended by ultrasonication in 0·3 mL physiological saline containing 0·01% Tween 80 and then were injected over 20 s into the left cardiac ventricle. During, and for 70 s after, the microsphere injection blood was withdrawn from a femoral artery at 0·5 mL min^{-1} by a syringe pump (Perfusor IV; Braun, Melsungen, FRG). The circulation was then stopped with an air embolism and an injection of 5 μL of a 1% Evans Blue (Merck) solution was made through the i.c.v. cannula so that its position

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could be confirmed visually after removal of the brain. The organs were removed, weighed and placed in plastic vials for radioactivity counting in a Packard Autogamma 500 scintillation spectrometer. The radioactivity in the femoral blood sample was also determined and the cardiac output and tissue blood flows were determined by the method of McDevitt & Nies (1976). Total peripheral and organ vascular resistances were calculated assuming central venous pressure to be zero; total peripheral resistance is given by (mean central arterial pressure/cardiac index) and organ resistance by (mean central arterial pressure/organ blood flow per g tissue).

Perfusion of the cerebrovascular system with melittin. Rats were anaesthetized as before, the right common carotid artery was cannulated for the recording of blood pressure and the animal placed in the stereotaxic frame. An inflow cannula was inserted into the left lateral cerebral ventricle as described above and outflow from the ventricular system was facilitated by drilling the occiput and piercing the atlanto-occipital membrane. The cerebral ventricles were perfused, using a Braun Perfusor IV syringe pump, at 26 μL min^{-1} with artificial cerebrospinal fluid containing melittin (Sigma) at a concentration of 37 μM. This rate of perfusion gave a perfusion pressure of 93 ± 13 mmH_2O (n = 15); this compares with cerebrospinal fluid pressures previously determined in the rat of $34{\cdot}5 \pm 23{\cdot}8$ mmH_2O (Hayes & Corey 1970) and 64 ± 2 mmH_2O (Mandell & Zimmerman 1980). Using Evans Blue dye it was found that the period between fluid entering and it leaving the ventricular system was 2 min.

Statistics. Values are given as mean $\pm$ s.e.m. In the bradykinin experiments between group comparisons were made by Student's unpaired *t*-test and within group comparisons by Student's paired *t*-test. In the melittin experiments, changes in blood pressure were compared by one-way analysis of variance.

Results

Preliminary experiments were carried out in order to determine the amount of bradykinin needed to produce a consistent response. It proved not to be possible to construct full dose-response curves in individual animals because the responses showed considerable desensitisation and over 30 min had to be left between doses in order to obtain consistent responses to any given dose. Consequently, rats received only 2 or 3 doses with 35–45 min between each administration. Below 10 μg, these responses varied for any given dose, commonly being biphasic (a depressor response developing within 1 min and lasting approximately 2 min was followed by a sustained pressor response lasting up to 15 min) but occasionally a monophasic pressor response was seen. In three animals given 1 μg, there were two which showed falls in mean arterial pressure of 15 and 12 mmHg and one had no depressor phase at all; the mean pressor response was 14 ± 1 mmHg. 30 μg bradykinin was found to give a consistent, maximal pressor response and was used to determine the effects of the peptide, given i.c.v., on haemodynamics.

Table 1. Tissue vascular resistances and blood flows in anaesthetized rats given 10 μL artificial cerebrospinal fluid (a.c.s.f.) or 30 μg bradykinin i.c.v.

	Vascular resistance (mmHg mL^{-1} min^{-1} g^{-1})		Blood flow (mL min^{-1} g^{-1})	
Tissue	a.c.s.f.	Bradykinin	a.c.s.f.	Bradykinin
Heart	$22{\cdot}6 \pm 4{\cdot}0$	$24{\cdot}2 \pm 2{\cdot}6$	$3{\cdot}92 \pm 0{\cdot}48$	$5{\cdot}11 \pm 0{\cdot}76$
Lungs	129 ± 48	180 ± 37	$1{\cdot}15 \pm 0{\cdot}27$	$0{\cdot}75 \pm 0{\cdot}12$
Liver[a]	355 ± 57	245 ± 16*	$0{\cdot}32 \pm 0{\cdot}05$	$0{\cdot}48 \pm 0{\cdot}03$**
Kidneys	$21{\cdot}8 \pm 1{\cdot}5$	$26{\cdot}7 \pm 2{\cdot}7$	$4{\cdot}60 \pm 0{\cdot}16$	$4{\cdot}45 \pm 0{\cdot}37$
Brain	190 ± 16	164 ± 16	$0{\cdot}54 \pm 0{\cdot}04$	$0{\cdot}74 \pm 0{\cdot}10$*
Testes	494 ± 38	569 ± 46	$0{\cdot}21 \pm 0{\cdot}02$	$0{\cdot}21 \pm 0{\cdot}02$
Epidid.	603 ± 103	872 ± 137	$0{\cdot}20 \pm 0{\cdot}04$	$0{\cdot}15 \pm 0{\cdot}03$
Skel. muscle[b]	516 ± 105	504 ± 93	$0{\cdot}25 \pm 0{\cdot}02$	$0{\cdot}28 \pm 0{\cdot}06$
Skin	706 ± 85	1397 ± 223**	$0{\cdot}15 \pm 0{\cdot}02$	$0{\cdot}09 \pm 0{\cdot}02$*
Spleen	$93{\cdot}0 \pm 11{\cdot}7$	210 ± 59*	$1{\cdot}18 \pm 0{\cdot}18$	$0{\cdot}70 \pm 0{\cdot}12$*
Stomach	214 ± 36	366 ± 48**	$0{\cdot}53 \pm 0{\cdot}09$	$0{\cdot}35 \pm 0{\cdot}05$
S. intest.	$66{\cdot}3 \pm 4{\cdot}2$	$73{\cdot}8 \pm 12{\cdot}5$	$1{\cdot}53 \pm 0{\cdot}11$	$1{\cdot}74 \pm 0{\cdot}25$
L. intest.	159 ± 14	240 ± 24**	$0{\cdot}65 \pm 0{\cdot}06$	$0{\cdot}51 \pm 0{\cdot}07$
Panc./mesent.	146 ± 17	269 ± 46*	$0{\cdot}75 \pm 0{\cdot}12$	$0{\cdot}50 \pm 0{\cdot}09$

[a] Hepatic artery. [b] Pectoral muscle. n = 6 for each group.
Statistically different from the saline group as determined by Student's unpaired *t*-test: * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

In six rats, i.c.v. injection of 30 μg bradykinin significantly increased mean arterial blood pressure from 99 ± 3 to 114 ± 3 mmHg ($P < 0{\cdot}001$; paired *t*-test) but heart rate was unaffected being 384 ± 11 beats min^{-1}, before, and 381 ± 11 beats min^{-1}, after bradykinin. Pulse pressure was unchanged, before and after the injection it was 59 ± 6 and 61 ± 6 mmHg, respectively. Injection of 30 μL artificial cerebrospinal fluid into six rats had no effect on either mean systemic blood pressure (103 ± 5 and 99 ± 5 mmHg before and after injection, respectively) or heart rate (392 ± 17 beats min^{-1}, before, and 391 ± 17 beats min^{-1} after injection).

Cardiac index was not different in the bradykinin group, in which it was $28{\cdot}1 \pm 2{\cdot}6$ mL min^{-1}/100 g body weight, from that of $30{\cdot}8 \pm 3{\cdot}3$ mL min^{-1}/100 g body wt in the control group. Total peripheral resistance was significantly higher ($P < 0{\cdot}05$; unpaired *t*-test) in the bradykinin group ($4{\cdot}23 \pm 0{\cdot}29$ mmHg mL^{-1} min/100 g body wt) compared to the control group ($3{\cdot}40 \pm 0{\cdot}33$ mm Hg mL^{-1} min/100 g body wt).

Table 1 shows that, relative to the controls, calculated tissue vascular resistance was significantly greater in the bradykinin group in the skin, spleen, stomach, large intestine and the pancreas/mesentery by 98, 126, 71, 51 and 84%, respectively. Only in the hepatic artery was resistance to blood flow less in the bradykinin group (by 31%) than in the rats given artificial cerebrospinal fluid i.c.v. The Table also shows that, in the bradykinin group, as a result of the larger changes in vascular resistance than in the other tissues, blood flow was significantly lower in both the spleen (by 41%) and the skin (by 40%). However, in the experimental animals blood flow was greater in the hepatic artery (by 50%) and brain (by 37%) than in the controls.

Infusion of 37 μM melittin into the cerebral ventricles of five rats at a rate of 26 μL min^{-1} caused an initial hypotensive response in which mean arterial pressure fell from 87 ± 7 to 77 ± 6 mmHg and which was maximal at $4{\cdot}2 \pm 2$ min (range 2–13 min) from the start of the infusion. This was followed by an increase in systemic blood pressure to 118 ± 4 mmHg which was relatively well-sustained and was reached at 20 ± 5 min (range 10–39 min) from the start of the infusion. Pulse pressures did not significantly change during the melittin infusion being 41 ± 4 mmHg before the infusion was started, 37 ± 5 mmHg at the maximum depressor point and 48 ± 5 mmHg at the maximum pressor response. Heart rate did not change significantly during the depressor phase but did show an increase above the pre-infusion level in the pressor phase; the values were 387 ± 13 beats min^{-1} before the infusion, 377 ± 8 beats min^{-1} at time of the maximum depressor response and 419 ± 13 beats min^{-1} during the pressor response; this last value was significantly greater than the pre-mellitin value ($P < 0{\cdot}05$; analysis of variance). Infusion of artificial cerebrospinal fluid alone had no significant effect on blood pressure. Infusion of 70 μM melittin at the same rate into three rats caused effects very similar to those of the lower dose.

Discussion

Intracerebroventricular administration of 30 μg bradykinin caused a pressor response of 15 mmHg which developed over the

course of 2 min and then was well-sustained. In the microsphere experiments no response was followed for more than 5 min but the preliminary experiments showed the response not to decline in animals which were followed for up to 20 min after the bradykinin was given. Pearson et al (1969) reported centrally-administered bradykinin to give biphasic responses but Corrêa & Graeff (1975) showed effects similar to those we found for the dose used in the microsphere experiments; there was an increase in mean arterial pressure of approximately 20 mmHg, no change in heart rate and no change in pulse pressure. In our experiments, bradykinin sometimes gave biphasic responses but only at the lower doses (0·1–3 μg) used. Pearson et al (1969) used a dose of 1 μg and suggested, on the basis of intravenously administered antagonists, that two peripheral mechanisms were responsible for the biphasic response since, although both phases were blocked by hexamethonium, the depressor phase could be blocked with phentolamine and the pressor phase with propranolol. These workers suggested that the pressor response could be due to stimulation of cardiac β-adrenoceptors but this seems unlikely since we found no change in cardiac output or heart rate during the pressor response to 30 μg; it is therefore possible that the pressor response is the result of sympathetic nervous stimulation of the renal juxtaglomerular apparatus.

The increase in blood pressure produced by i.c.v. bradykinin was due to changes in vascular resistance rather than cardiac performance. The tissue vascular resistances show that, with bradykinin, there were significant increases in organ resistance in the spleen, stomach, large intestine and pancreas/mesentery and a significant decrease in the hepatic artery. Hence, it would seem that much of the peripheral resistance increase is in the gastrointestinal tract with the spleen and the skin also making contributions. Since there was no significant change in resistance, the changes in blood flow in the brain appear to be due to a relative rather than an absolute change in vascular resistance.

It is possible that the change in blood flow in the hepatic artery is locally rather than centrally-mediated. When vascular resistance increases in the gastrointestinal tract, there is a compensatory decrease in hepatic arterial resistance (Hanson & Johnson 1966), even in isolated livers (Sato et al 1977) and this phenomenon possibly explains the change in hepatic arterial blood flow.

The experiments with melittin were intended to try to determine whether or not there might be cardiovascular effects arising from stimulation of membrane-bound, endogenous kallikrein in the central nervous system of the rat. Melittin gave a biphasic change in blood pressure, similar to those seen with the lower doses of bradykinin. This contrasts with the dog in which i.c.v. infusion of melittin gave only a monophasic increase in blood pressure which was accompanied by an increase in heart rate (Thomas et al 1984). The present results in the rat also show an increase in heart rate, an effect which was not seen with bradykinin along although Diz & Jacobowitz (1984) produced changes in heart rate on local injection of bradkinin into hypothalamic nuclei. The differential effects of melittin and bradykinin alone although Diz & Jacobowitz (1984) produced changes in heart rate on local injection of bradykinin into not be the only effect of this component of bee venom when given i.c.v. Also, larger blood pressure changes occurred after melittin and thus it is not possible to use the present data to give unqualified support to the hypothesis that there is a functional kallikrein-kinin system involved in the central control of the cardiovascular system. Nevertheless, the results confirm that bradykinin can act centrally to increase systemic blood pressure and show that this is the result of increased peripheral vascular resistance.

This work was supported by a grant from the British Heart Foundation (No. 84/49)

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