The effect of a bradykinin antagonist on vasodilator responses with particular reference to the submandibular gland of the cat

S. Barton, E. Karpinski and M. Schachter[a]

Department of Physiology, University of Alberta, Edmonton (Alberta, Canada T6G 2H7), and [a]Department of Chemistry, University College London, London WC1H OAJ (England)
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Summary. A bradykinin analogue, D-Arg[Hyp^3, Thi^{5,8}, D-Phe^7]-Bk, antagonized the vasodilator effect of bradykinin injected close-arterially in the submandibular salivary gland of the cat, without affecting that due to acetylcholine or nerve stimulation. The same analogue also antagonized the hypotensive response to bradykinin injected intravenously in cats and rabbits. We conclude that functional hyperaemia in the submandibular gland of the cat is not due to the release of bradykinin by salivary kallikrein.
Key words. Bradykinin; bradykinin antagonists; vasodilatation; submandibular salivary gland.

Several bradykinin (Bk) analogues have recently been synthesized and shown to act as competitive and specific antagonists of bradykinin and related kinins[1]. Two Bk receptor classes were postulated earlier and designated as B_1 and B_2 receptors[2]. The new Bk antagonists, however, appear to be specific for the B_2 receptors[1,3-5] which are far more common than the B_1 type. The latter are present mainly in the isolated aorta and mesenteric vein of the rabbit, and are induced after incubation of the tissue in vitro. The most specific B_1 antagonist is [Leu^8] des-Arg^9 Bk^6.

The new B_2 receptor antagonists are analogues of Bk with D-phenylalanine substituted for proline at position 7; further increases in potency have been obtained by additional substitutions at positions 5 and 8[1].

The availability of these B_2 antagonists led us to use one of them in a crucial test of whether or not bradykinin is the metabolic mediator of the physiological vasodilatation in the submandibular gland of the cat, an old but unresolved problem[7-9]. We also examined its ability to antagonize the hypotensive effect of Bk on the arterial blood pressure of the cat and rabbit. The B_2 antagonist used in all experiments was D-Arg[Hyp^3, Thi^{5,8}, D-Phe^7]-Bk and was generously provided by Drs J. M. Stewart and R. Vavrek.

Methods and materials. Cats of either sex (2.5–5.3 kg, n = 12) were starved overnight and then anaesthetized with chloralose (200–260 μM kg^{-1} i.v.). Rabbits (male, 2.2–3.6 kg, n = 3) were anaesthetized with urethane (10–15 mM kg^{-1} i.v.). Blood flow through the submandibular gland of the cat was measured using a forced convection flowmeter with a probe in the external jugular vein[8]. Stimulation of the parasympathetic (chorda-lingual) and sympathetic nerves to the submandibular gland and blood pressure measurements were carried out as described previously[8]. Drugs were dissolved in 0.15 M NaCl or in 0.15 M NaCl containing 0.5% bovine serum albumin and injected either close-arterially via the lingual artery (in a volume of 0.5 ml over 20 s), or via a femoral vein (0.5–1.0 ml as a bolus). The drugs and chemicals used were acetylcholine chloride (ACh), bradykinin triacetate, α-chloralose, heparin, vasoactive intestinal peptide (VIP) (Sigma), bovine serum albumin (Calbiochem) and urethane (Fisher Scientific Company). The Bk antagonist used was D-Arg[Hyp^3, Thi^{5,8}, D-Phe^7]-Bk, also referred to as compound B4162.

Effect of bradykinin antagonist (B4162) on vasodilator responses to bradykinin (Bk) and nerve stimulation in the submandibular gland of the cat. Stimulation of the chorda-lingual (parasympathetic) nerve (8V, 0.5 ms, 10 Hz, 1–30 s) caused salivation and a marked increase in blood flow. Bradykinin (0.1–0.5 nmole i.a.) also caused an increase in blood flow but it was usually slower in onset and more prolonged. A bolus injection of Bk antagonist B4162 (4–400 nmole i.a.) usually had no effect on blood flow but occasionally caused a small and transient increase. However, whereas the effect of Bk was greatly reduced or abolished after injection of B4162, that of nerve stimulation was unaffected. The results of a single typical experiment are shown in figure 1. In this experiment, compound B4162 (50 nmole i.a.) abolished the response to Bk (0.1 nmole i.a.) but had no effect on the response to chorda-lingual nerve stimulation. Similar experiments were carried out on 7 cats (as shown in fig. 2). One minute after injection of the antagonist the mean response to Bk fell to 9% of the control. Recovery from the antagonist was gradual so that 15 and 30 min later the mean responses to injected Bk rose to 42 and 71% of the control

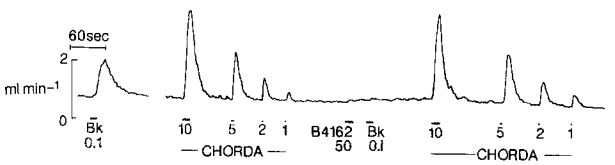

Figure 1. Cat 4.2 kg, ♂. Blood flow through the cat submandibular gland, showing the vasodilator response to bradykinin (Bk) and parasympathetic nerve stimulation (chorda), before and after injection of a Bk antagonist (B4162). Bk and B4162 were injected close-arterially into the gland (doses given in nmole); duration of nerve stimulation given in seconds. Blood pressure (not shown) remained constant at approximately 15.5–16.5 kPa.

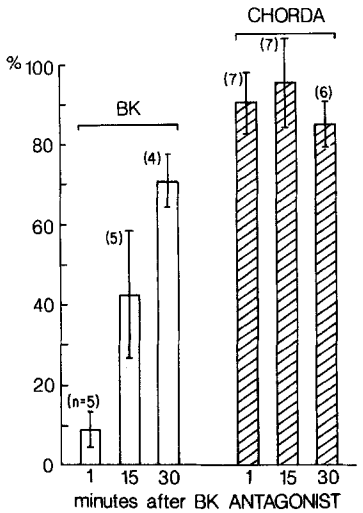

Figure 2. The effect of a bradykinin antagonist (B4162, 50 nmole i.a.) on the vasodilator responses to bradykinin (Bk, 0.1 nmole i.a.) and to parasympathetic nerve stimulation (chorda, 5 s), 1, 15 and 30 min after the antagonist was given. The vasodilator responses are expressed as a percentage of the control response before the antagonist. The bars are the mean values of 4–7 observations obtained in experiments on seven cats; the vertical lines represent SE mean.

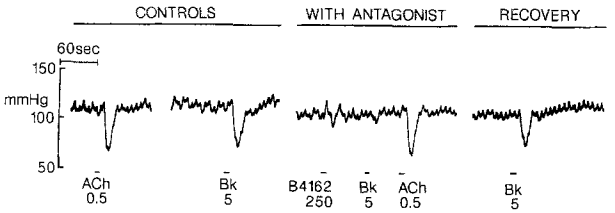

Figure 3. Cat 4.3 kg ♀. Hypotensive effect of acetylcholine (ACh, 0.5 nmole i.v.) and bradykinin (Bk, 5 nmole i.v.) before and immediately after injection of Bk antagonist (B4162, 250 nmole, i.v.). Partial recovery occurred 5.5 min after injection of the antagonist.

values, respectively. The results of these seven experiments are shown in figure 2. It shows that the Bk antagonist B4162 (50 nmole i.a.) greatly reduced the vasodilator response to Bk (0.1 nmole i.a.) but had no effect on chorda-lingual nerve stimulation (5 s). The same result was obtained if the nerve was stimulated for 1, 2 or 10 s. (not shown in the figure). The vasodilator responses to ACh injected close-arterially and to sympathetic nerve stimulation (an 'after' dilatation) were also unaffected by the Bk antagonist.

Effect of bradykinin antagonist (B4162) on hypotensive responses to bradykinin. Bk (1–100 nmole i.v.) caused a fall in arterial blood pressure in cats (n = 4) and rabbits (n = 3). The hypotensive response was reduced in a dose-dependent manner by the Bk antagonist. The molar ratio of antagonist to agonist resulting in 50% reduction of the Bk response was 2.4, 6 and 9 in three experiments in cats, and 1.8 and 3 in two experiments in rabbits. The antagonism was temporary, with 50% recovery from complete inhibition in 2–6 min. Similar results have also been obtained in guinea-pigs (Barton, Padsha and Schachter, unpublished). Figure 3 shows a typical result of an experiment in a cat in which the Bk antagonist B4162 (250 nmole i.v.) abolished the hypotensive response to Bk (5 nmole i.v.) but had no effect on that to ACh (0.5 nmole i.v.). The specificity of B4162 was further demonstrated in other experiments (2 cats, 1 rabbit) in which it was shown to have no effect on the hypotensive responses to VIP (1–5 nmole i.v.) and substance P (5 nmole i.v.).

Discussion. Our results show that it is possible to abolish the vasodilatation produced by bradykinin injected close-arterially into the submandibular gland of the cat whilst that produced by nerve stimulation is unaffected. This confirms the view that the kallikrein-kinin system does *not* mediate functional hyperaemia in the cat submandibular gland [8, 9]. Studies carried out on the submandibular gland of the rat, which has one of the highest concentrations of tissue kallikrein in mammalian organs, led to the conclusion that in this species salivary kallikrein mediates the nerve-induced vasodilatation [10]. In a recent report these authors found that the intra-arterial infusion of the same Bk antagonist used in our studies, blocked the vasodilator response of the rat submandibular gland to Bk [11]. The vascular response to nerve stimulation, however, was not tested in the latter experiments. Further studies are still needed to clarify the significance of salivary kallikrein and of the kinin which it can release.

These new Bk antagonists are likely to prove most valuable. The synthesis of analogues with longer duration of action would make them even more useful for investigating the significance of Bk in physiological and pathophysiological conditions. There is also reason to expect that they will have some therapeutic applications in humans.

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Corticosteroid effects on cholinergic enzymes in ethanol-treated fetal brain cell cultures

J. W. Brown[a], A. D. Okonmah[b], K. F. A. Soliman[c], A. Carballeira[a] and L. M. Fishman[a]

[a] *Medical Research Service, Veterans Administration Medical Center and Department of Medicine, University of Miami School of Medicine, Miami (Florida 33125, USA),* [b] *College of Pharmacy, Florida A & M University Clinical Research Unit, P.O. Box 421091, Miami (Florida 33142, USA), and* [c] *College of Pharmacy, Florida A & M University, Tallahassee (Florida 32307, USA)*

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Summary. In the presence of ethanol, corticosterone and dexamethasone inhibit choline acetyltransferase and acetylcholinesterase activities in cultured fetal brain cells of the rat. These results suggest that corticosteroids may have an important influence on the activity of cholinergic enzymes in the fetal brain and may antagonize the effects of ethanol in this setting.

Key words. Fetal brain cells; ethanol; corticosterone; dexamethasone; choline acetyltransferase; acetylcholinesterase.

Recent studies have demonstrated stimulatory effects of ethanol (ETOH) on cholinergic enzymes in brain cells of the fetal rat [1]. Combined in utero and in vitro ethanol exposure has been found to result in elevations of both choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities. These effects could contribute to the cognitive and neu-