Characterisation of Smooth Muscle $\alpha$-Adrenoceptors and of Responses to Electrical Stimulation in the Cat Isolated Perfused Middle Cerebral Artery

I. C. Medgett and S. Z. Langer

Department of Biology, Laboratoires d'Etudes et de Recherches - Synthélabo, 58, rue de la Glacière, F-75013 Paris, France

Summary. The effects of periartrial electrical stimulation and of drugs with $\alpha$-adrenoceptor agonist or antagonist activity were examined in cat isolated perfused middle cerebral arteries, in order to characterise the smooth muscle adrenoceptors. The perfused vessel preparation was stable and did not develop intrinsic tone when perfused at 5 ml/min with Krebs' solution. Exogenous noradrenaline caused increases in perfusion pressure, the pD$_2$ value being 6.8 ± 0.1. The maximum effect of noradrenaline was only 22 ± 4% of that of KCl (127 mM). Adrenaline was equipotent with noradrenaline whereas dopamine and phenylephrine were approximately 100 times less potent. The $\alpha_2$-adrenoceptor antagonist RX 781094 (0.1–10 $\mu$M) caused concentration-dependent rightward shifts in the noradrenaline concentration-response curve (in the presence of 4 $\mu$M cocaine and 1 $\mu$M propranolol), although the antagonism was not of a simple competitive nature. Prazosin (0.3 $\mu$M) failed to produce a significant shift to the right in the concentration effect curve to noradrenaline; however, the effects of maximal concentrations of noradrenaline were slightly but significantly reduced. If tone was induced in the vessels, tetrodotoxin-sensitive dilatations could be produced by electrical stimulation. In the absence of tone, the same stimulation parameters were ineffective in causing vasoconstriction. Increasing pulse width or voltage led to substantial vasoconstrictor responses, which were resistant to tetrodotoxin. These results demonstrate that in a cerebral artery which does not show a sympathetic vasoconstrictor response to electrical stimulation, both $\alpha_1$- and $\alpha_2$-adrenoceptors appear to be present on smooth muscle cells although the responses to exogenous noradrenaline are predominantly mediated by the $\alpha_2$-adrenoceptor subtype.

Key words: Smooth muscle $\alpha$-adrenoceptors - $\alpha_1$- and $\alpha_2$- Adrenoceptor subtypes - Neurogenic vasodilatation - Non-neurogenic vasoconstriction - Cat isolated perfused cerebral arteries

Introduction

There is now considerable evidence to suggest that at least in vivo vascular smooth muscle $\alpha$-adrenoceptors may be subclassified into $\alpha_1$- and $\alpha_2$-type adrenoceptors, based on relative potencies of selective agonists and antagonists (Drew and Whiting 1979; Docherty and McGrath 1980; Langer et al. 1980; reviewed by Langer and Shepperson 1982, and by McGrath 1982). However, the corresponding in vitro data, essential for a thorough and complete pharmacological analysis, is more meagre. Canine venous smooth muscle in vitro (saphenous vein segments) appears to contain both $\alpha_1$- and $\alpha_2$-adrenoceptor subtypes (de Mey and Vanhoucke 1981; Shepperson and Langer 1981). In vitro evidence for vasoconstrictor $\alpha_2$-adrenoceptors in arterial smooth muscle is less convincing. Only two reports have appeared suggesting the existence of arterial smooth muscle $\alpha_2$-adrenoceptors: in isolated cerebral arteries of the dog (Sakakibara et al. 1981) and cat (Skärby et al. 1981). In both these studies, the crucial evidence was based on the selective $\alpha_2$-antagonistic effect of yohimbine; however, in the study by Sakakibara et al. (1981), yohimbine, in low concentrations, had a contractile effect by itself, and in the study by Skärby et al. (1981), marked reductions in the maximum response to noradrenaline were observed. Thus, the question of the existence of $\alpha_2$-adrenoceptors in arterial smooth muscle in vitro remains controversial.

In the present study, we have attempted to characterise the $\alpha$-adrenoceptors in the smooth muscle of the cat middle cerebral artery in more detail. As selective $\alpha_1$- and $\alpha_2$-adrenoceptor antagonists, we have used prazosin and RX 781094 (Chapleo et al. 1981), respectively. RX 781094 is a new, highly potent, synthetic $\alpha_2$-adrenoceptor antagonist with considerably greater $\alpha_2$/$\alpha_1$ selectivity than yohimbine, and is the best compound currently available in this respect. Since the stability and sensitivity of commonly used in vitro preparations of cerebral vessels (i.e. spiral strips or cylindrical segments) is often poor, we have used a technique for perfusion and superfusion of the vessels which appears to improve stability and also the sensitivity to contractile agents.

In view of the suggestion of Langer et al. (1980, 1981), that vascular smooth muscle $\alpha_2$-adrenoceptors occur mainly extrajunctionally (i.e. they do not mediate sympathetic vasoconstriction), we have also attempted to observe and characterise responses to periartrial electrical stimulation of cat middle cerebral arteries, which have a rather dense adrenergic and non-adrenergic innervation (see, e.g., Lee 1981). We report that, although both $\alpha_1$- and $\alpha_2$-adrenoceptors are present in the cat middle cerebral artery, vasoconstrictor responses to exogenous agonists are mainly mediated by the $\alpha_2$-adrenoceptor subtype. This finding is of particular interest in view of our inability to obtain a sympathetic vasoconstric- tor response to periartrial electrical stimulation in this preparation.

Some of the results of the present study have previously been communicated to the British Pharmacological Society (Langer and Medgett 1982).

Send offprint requests to S. Z. Langer at the above address
Materials and Methods

Cats of either sex weighing 2 - 3 kg were anaesthetised with pentobarbital sodium (30 mg/kg, i.p.), exsanguinated and the brain then rapidly removed and placed in a dish of warm oxygenated Krebs’ solution. Using a dissecting microscope, the proximal 5 cm of each cerebral artery was cannulated in situ with polyethylene tubing (0.7 mm O.D.), before removal from the brain surface. The arteries were mounted in a perfusion system and Krebs’ solution perfused through the proximal cannula. The distal end of the artery was left open and uncanulated and the Krebs’ solution thus allowed to superfuse the extraluminal surface. The artery was kept upright under a tension of about 0.25 g via a weighted tie on the distal end of the artery. The temperature of the Krebs’ solution was maintained at 37°C and pH 7.6 and gassed with a mixture of 5% CO₂ in O₂. The Krebs’ solution had the following composition (in mM): NaCl, 118; KCl, 4.7; NaHCO₃, 25; NaH₂PO₄, 1.0; MgCl₂, 1.2; CaCl₂, 1.3; glucose, 11.1, ascorbic acid, 0.1. The artery was perfused at a rate of 5 ml/min (LIN constant perfusion pump). In preliminary experiments this flow rate was shown to be fast enough that the vessels did not develop intrinsic tone (see Results); in addition, vasoconstrictor responses to KCl (127 mM; Skårby et al. 1981) were optimal. Vasoconstriction was measured as an increase in perfusion pressure at constant flow, using a Statham 23 Db pressure transducer. A 30 min stabilisation period was allowed before responses to drugs or to electrical stimulation were obtained. During this period resting perfusion pressure fell from an initial level of 50 - 100 mm Hg to less than 10 mm Hg and, thereafter, remained essentially constant. Routinely, at least two responses to KCl (127 mM) were then obtained at 15 min intervals until they were constant. Drugs were given by momentarily stopping the perfusion medium and replacing the Krebs’ solution with one containing the required concentration of the drug, which was subsequently perfused until the maximum response was obtained, before returning to drug-free Krebs’ solution. To obtain a stable basal perfusion pressure, such that increases of 1 - 2 mm Hg could be accurately measured, it was found necessary to include a filter and a bubble trap in the perfusion line between the Krebs’ reservoir and the pump. Periarterial electrical stimulation was applied through bipolar platinum ring electrodes using monophasic square wave pulses, which were monitored occasionally with an oscilloscope. Other stimulation parameters are given in Results.

For the contractile agonists, non-cumulative concentration-response curves were obtained using randomised single-concentration perfusions (see Results). When antagonists were used, they were infused at least 20 min before the effect of an agonist was reassessed. Similarly, individual rather than cumulative frequency response curves to electrical stimulation were obtained, the order of stimulation frequencies being randomised. In most cases, responses were expressed as percentages of the increase in perfusion pressure elicited by KCl in the particular experiment.

The following drugs were used: l-adrenaline bitartrate (Sigma, St. Louis, MO, USA); atropine sulphate (Sigma, St. Louis, MO, USA); bretylium tosylate (Wellcome, Beckenham, Kent, UK); cirazoline hydrochloride (Synthelabo, Paris, France); cocaine hydrochloride (Cooperation Pharmaceutique Française, Paris, France); dopamine hydrochloride (Sigma, St. Louis, MO, USA); guanethidine sulphate (Ciba, Basel, Switzerland); indomethacin (Sigma, St. Louis, MO, USA); l-noradrenaline bitartrate (Sigma, St. Louis, MO, USA); papaverine hydrochloride (Sigma, St. Louis, MO, USA); phen tolamine mesylate (Ciba, Basel, Switzerland); l-phenylephrine hydrochloride (Sigma, St. Louis, MO, USA); prazosin hydrochloride (Pfizer, Croton, CT, USA); d,l-propranolol hydrochloride (Sigma, St. Louis, MO, USA); prostaglandin F₂α Tris salt (Sigma, St. Louis, MO, USA); reserpine (Sigma, St. Louis, USA); RX 781094 hydrochloride (2-[2-(1,4-benzodioxanyl)]-2-imidazoline hydrochloride); Reckitt and Colman, Hull, UK); serotonin creatinine sulphate (Sigma, St. Louis, MO, USA); tetrodotoxin (Sigma, St. Louis, MO, USA). The salts were dissolved in distilled water, which in the case of the catecholamines contained 50 μg/ml ascorbic acid to prevent oxidation of the catechol groups. For prazosin, it was necessary to effect dissolution (4.2 mg in 10 ml distilled water) using an ultrasonic vibrator (Ultrasound Annemasse) in order to avoid using solvents (dimethyl acetate or lactic acid) which have been shown to cause a depression of the maximum response to noradrenaline in cat middle cerebral arteries (Skårby et al. 1981). Suspensions of reserpine base (3 mg in 3 ml) were prepared for i.p. injection, in 0.9% saline solution. Indomethacin was dissolved in absolute ethanol. Tetrodotoxin was dissolved in distilled water to give a 1 mM stock solution in citrate buffer. Statistical analysis was performed using Student’s t-test for paired or unpaired data, as appropriate.

Results

Effects of Contractile Agonists on Cat Middle Cerebral Arteries

The response to a maximally effective concentration of KCl (127 mM) in isolated cat middle cerebral arteries was an increase in perfusion pressure of 108 ± 4 mm Hg (n = 70). This response was essentially monophasic and well maintained (see Figs. 1, 7a, 8 and 9), and thus appears to differ from that of rabbit perfused middle cerebral arteries which show an initial phasic contraction followed by a smaller tonic contraction when exposed to KCl (Oudart et al. 1981). Routinely, responses to noradrenaline (10 μM; Fig. 1) were elicited until they became constant (usually 3 or 4 responses). Subsequently, concentration-response curves to noradrenaline were obtained. The sensitivity of the preparation to noradrenaline slightly but progressively increased for 2 - 3 h after which time the responses to noradrenaline remained constant for a further 3 - 4 h, as determined in control experiments. After constant responses had been obtained, either an antagonist was perfused and responses to noradrenaline again elicited, or a different agonist was applied (see below). Figure 1 illustrates that noradrenaline responses (0.01 - 100 μM) developed more slowly than those to KCl and were monophasic, showing a single clear plateau. In close agreement with Skårby et al. (1981), the maximum response to noradrenaline at the time of development of maximum sensitivity was 22 ± 4% of that of KCl (n = 12). The pD₂ value for noradrenaline was 6.8 ± 0.1 and the threshold concentration was around 1 nM (see Fig. 2). Concentrations of noradrenaline in excess of 100 μM were not routinely employed since desensitisation of the arteries to the subsequent effects of the lower noradrenaline concentrations (1 nM - 0.1 μM) appeared to occur. Figure 2 also shows the concentration-response curve obtained for noradrenaline in