Br. J. Pharmac. (1985), **86**, 217–227

Electrophysiological analysis of the nature of adrenoceptors in the rat basilar artery during development

N.G. Byrne, G.D.S. Hirst*[1] & W.A. Large

Department of Pharmacology, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE and Department of Pharmacology*, John Curtin School of Medical Research, The Australian National University, Canberra, A.C.T., Australia

1 The nature of adrenoceptors in basilar arteries of neonatal rats was investigated by means of electrophysiological techniques.

2 In immature (2–6 day postnatal) rats, micro-injection of noradrenaline elicited a depolarization which consisted of two components. The initial 'fast' component (time to peak of 0.3–4 s) was slightly reduced by phentolamine and was not antagonized by propranolol. The second 'slow' component (time to peak of about 50 s) was not blocked by phentolamine but was antagonized by low concentrations (10^{-7} M) of propranolol.

3 In immature rats, micro-injection of isoprenaline was more potent than noradrenaline in evoking the 'slow' depolarization but less effective in eliciting the 'fast' response. The pharmacology with respect to adrenoceptor antagonists of both components of the isoprenaline- and noradrenaline-induced depolarizations was similar. There was some evidence of inhibitory β-adrenoceptors in immature rat basilar vessels.

4 In adult rats (6 week old) noradrenaline produced a large 'fast' depolarization which was followed by a 'slow' tail response. Both components were not antagonized by phentolamine or propranolol.

5 It appears that in the basilar artery of neonatal rats there are excitatory α- and inhibitory β-adrenoceptors but the major responses to noradrenaline and isoprenaline are mediated by γ- and excitatory β-receptors. In adult animals the γ-adrenoceptor predominates.

6 Experiments were carried out in which agonists were applied by ionophoresis. These results confirm the presence of excitatory β-receptors in neonatal basilar vessels and show the response has slow kinetics and it is likely that the β-receptors are distributed uniformly over the smooth muscle surface. In adult animals it was not possible to elicit an excitatory β-receptor-mediated response.

7 The ionophoretic application of noradrenaline never evoked a perceptible depolarization which could be attributed to γ-adrenoceptor stimulation. This result is discussed in terms of receptor distribution with respect to synaptic function in a syncytium.

Introduction

Most systemic and cerebral arteries and arterioles are innervated by sympathetic nerves which show a characteristic catecholamine fluorescence after appropriate fixation when viewed with ultraviolet light (Norberg & Hamberger, 1964). Stimulation of such nerves results in vasoconstriction in most vascular beds (Folkow & Neil, 1971). In adult systemic and cerebral arteries, unlike the pulmonary artery and many veins, stimulation of the sympathetic nerves evokes a rapid depolarization and this excitatory junction potential (e.j.p.) is resistant to α-adrenoceptor antagonists (e.g. Hirst & Neild, 1980a). It was suggested that the e.j.p. recorded from these blood vessels resulted from activation of a novel type of adrenoceptor, the γ-receptor, which is localized intrajunctionally and that the α-adrenoceptor, which is activated usually by exogenous agonists, is situated in the extra-synaptic regions (Hirst & Neild, 1980b; Hirst *et al.*, 1982). However, during the development of

[1] Present address: Baker Institute, Alfred Hospital, Commercial Road, Prahan, Melbourne, Victoria.

innervation to systemic arteries the first clear electrical response (a slow depolarization), which can be attributed to sympathetic nerve stimulation, is antagonized by phentolamine and therefore presumably results from α-receptor activation. Some few days after the histological demonstration of synaptic structures the e.j.p. has a rapid time course and is not blocked by α-receptor antagonists (Hill *et al.*, 1983). Thus α-adrenoceptors appear first in systemic arterial smooth muscle and the junctional γ-receptor appears later in development after the synaptic structure has been established.

The experiments described in this paper were designed to examine the pharmacological properties of adrenoceptors present on the rat basilar artery during development. The basilar artery of mature rats appears to possess no (or negligible) α-adrenoceptors (Hirst *et al.*, 1982) and thus it seemed of interest to investigate whether α-receptors were present in vessels of neonatal rats and regressed or whether immature arteries were devoid of α-receptors. It became apparent during the course of the experiments that there are some α-adrenoceptors on neonatal rat basilar arterial smooth muscle but the main finding is that the major responses to noradrenaline are mediated by excitatory β- and γ-adrenoceptors. During development the excitatory β-receptors regress and the γ-adrenoceptors become predominant. A preliminary account of some of these results has already been published (Byrne *et al.*,1985).

Methods

Rats (of either sex) with postnatal ages from 2 days to 6 weeks were killed by decapitation. The basilar artery and the surrounding pia mater were dissected and pinned out for intracellular recording. The tissue was superfused continuously with Krebs solution at a rate of 1–3 ml per min (the bath volume was approximately 0.25 ml). Normal Krebs contained (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11 and was bubbled with 5% CO_2: 95% O_2. Experiments were carried out at room temperature (20–23°C) and at 33–35°C.

Membrane potentials were recorded with intracellular glass micro-electrodes filled with 0.5 M KCl with resistances 150–200 MΩ. Agonists were applied by three different methods: (a) bath application, (b) by micro-injection close to the tissue and (c) by ionophoresis. In the ionophoretic experiments electrodes similar to those used for recording membrane potential were filled with the appropriate drug solution at a concentration of 0.1 M and the agonists were ejected with a high voltage current pump (Purves, 1979). The technique of micro-injection (Byrne & Muir, 1985) was usually preferred to bath application because this method revealed a 'fast' γ-receptor-mediated response to noradrenaline which was not always apparent when the drug was included in the bathing fluid (a γ-receptor-mediated depolarization is resistant to both phentolamine and propranolol). For application by micro-injection, micro-syringes with needles of outside and inside diameters of about 0.5 mm and 0.15 mm respectively were used. Stock solutions of drugs (0.1 mM–1 M) were made up in 0.9% NaCl solution and volumes of 1–5 μl were injected directly into the organ bath with the needle tip 2–5 mm from the tissue. The injection took 2–3 s. Application of saline solution (not containing any drug) was tested frequently to ensure that membrane responses were elicited by the drug rather than by any mechanical disturbance or other artefactual influence.

The results from one such experiment are shown in Figure 1. Bath perfusion of noradrenaline 1 mM produced a slow depolarization of about 7 mV in

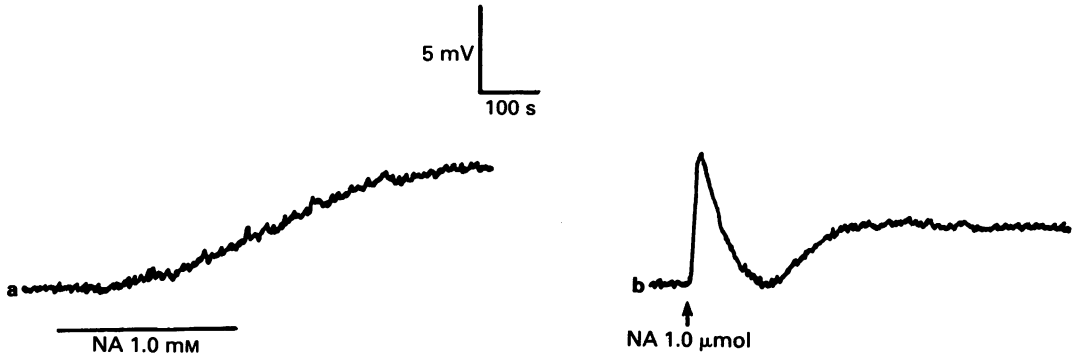

Figure 1 Comparison of the response of the rat basilar artery (6 weeks old) to noradrenaline (NA) added by bath perfusion (a) and by injection into the bath using a micro-syringe (b). Membrane potential (E_m): −54 mV. Room temperature, and 10^{-6} M phentolamine present.

amplitude (Figure 1a). However, when noradrenaline (1.0 μmol) was added by micro-injection, there was a slow depolarization which was preceded by a fast response of about 8 mV (Figure 1b). If the added noradrenaline by injection was uniformly distributed throughout the total bath volume, the concentration would reach about 4 mM, slightly higher than in the bath perfusion. However, as the injection was made some distance from the tissue (to avoid disturbing the micro-electrode) and the rate of perfusion was rapid, it is likely that the actual concentration of noradrenaline reaching the tissue is much lower than 4 mM but in any event the indisputable fact is that injection of noradrenaline evoked a biphasic depolarization whereas bath perfusion produced only a slow response. It should be noted that this experiment was carried out in an artery from a 6 week old rat in which there appear to be no α-adrenoceptors (Hirst *et al.*, 1982). It was possible to obtain consistent responses by this technique of micro-injection of agonists as long as the syringe needle was inserted at about the same position in the organ bath and the injection was made at the same rate. We have some evidence to suggest that the γ-adrenoceptor becomes desensitized during bath application, a similar desensitization to α-receptor activation also occurs in the anococcygeus muscle (Large, 1983). In some preparations (see later) it was possible to record reproducible γ-receptor mediated

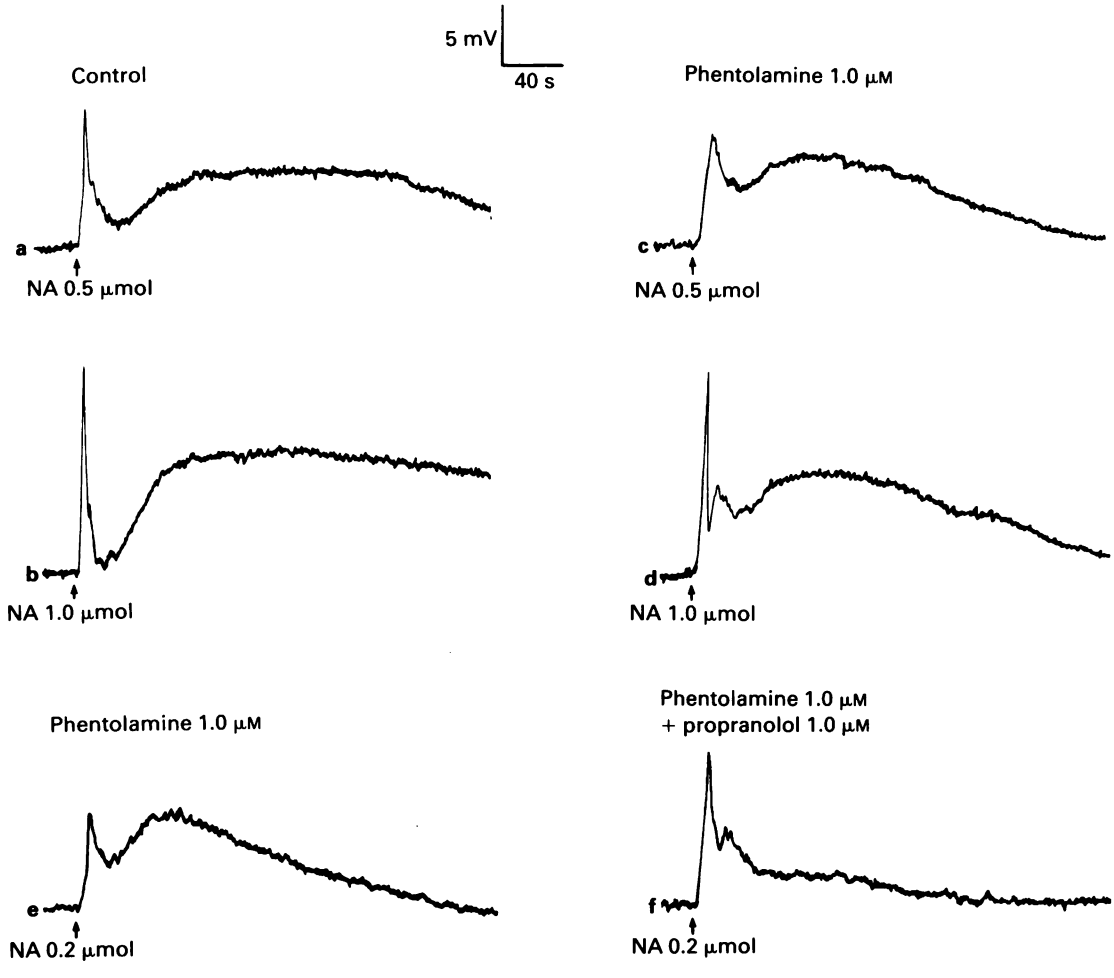

Figure 2 Effect of adrenoceptor antagonists on the depolarizations evoked by micro-injection of noradrenaline (NA) in the immature rat basilar artery. (a)–(d) were recorded from the same cell ($E_m = -48$ mV; 6 day old rat). (a) and (b), normal Krebs and (c) and (d), 10^{-6} M phentolamine present; (e) and (f) were recorded from another cell ($E_m = -58$ mV; 3 day old rat) and illustrate the effect of propranolol. Phentolamine 10^{-6} M was present in both (e) and (f). Room temperature.

responses to bath application of noradrenaline with concentrations as low at 5×10^{-7} M but the method of micro-injection was usually used. Antagonists were added to the bathing solution by changing the composition of the superfusion solution and allowing the tissue to equilibrate for a minimum of 10 min.

Excitatory junction potentials (e.j.ps) were evoked by field stimulation (pulse width = 0.03–0.1 ms) using Ag-AgCl electrodes placed on either side of the preparation.

The following drugs were used: noradrenaline bitartrate; isoprenaline sulphate; phentolamine mesylate; propranolol hydrochloride and methysergide bimaleate.

Results

Resting activity

At room temperature the preparations from both immature (2–6 days) or mature (4–6 weeks) rats usually showed no spontaneous electrical or mechanical activity but occasionally rhythmic oscillations in membrane potential which initiated action potentials were observed. This activity was more likely to occur if the preparation was warmed to 33–35°C.

The mean resting membrane potential recorded at room temperature was -50.3 ± 1.3 mV (mean $\pm$ s.e.mean, $n = 26$) for immature (2–6 days) preparations and -56 ± 1.5 mV for mature (4–6 weeks) preparations. Since it was occasionally possible to record values of up to -65 mV in the immature artery, the lower mean value obtained may reflect the difficulties of making recordings from these tissues rather than a true developmental change.

Responses to noradrenaline and isoprenaline in the basilar artery of immature (2–6 day postnatal) rats

In normal Krebs solution injection of noradrenaline (1 nmol–1 μmol) evoked a depolarization that comprised two components (Figure 2a and b): an initial 'fast' phase which peaked within 0.3–4.0 s followed by the second component, a 'slow' depolarization with a time to peak of 50 to 70 s. Phentolamine (10^{-6} M) reduced slightly the fast depolarization to submaximal doses of noradrenaline indicating that there was a small α-

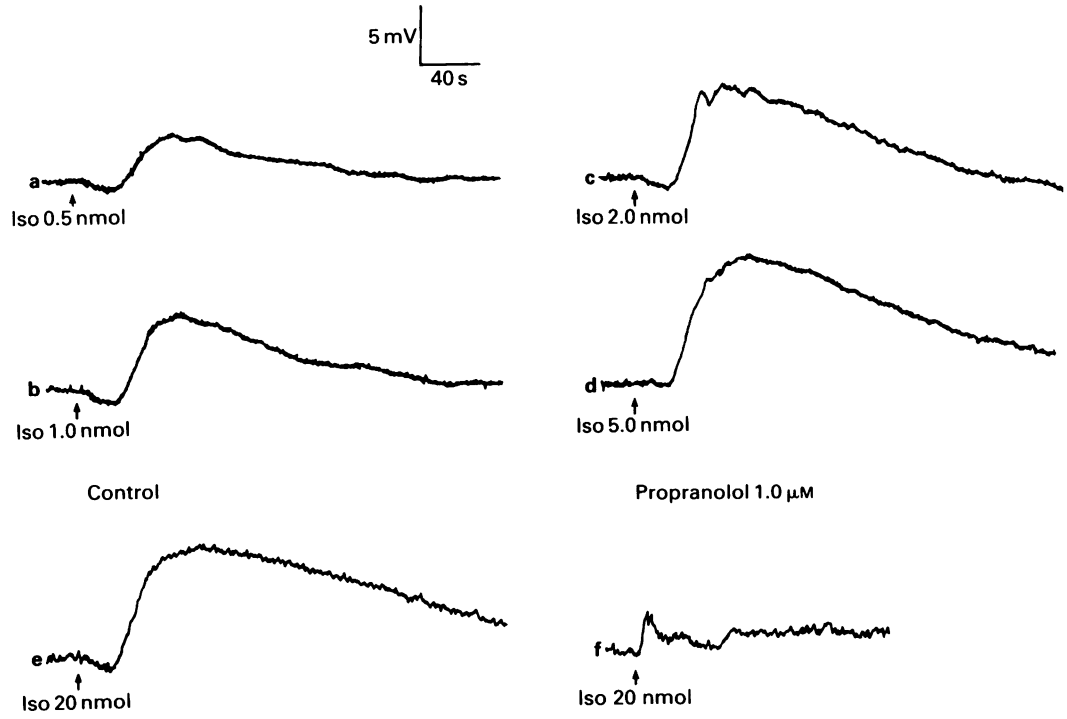

Figure 3 Depolarizations to isoprenaline in the immature rat basilar artery. Dose-dependent depolarization to isoprenaline are shown in traces (a)–(d) recorded from one cell ($E_m = -48$ mV; 6 day old rat). Note that hyperpolarizations precede the depolarizations. Records (e) and (f) were recorded from another cell ($E_m = -58$ mV; 3 day old rat) and illustrate the effect of propranolol on the response to isoprenaline. Phentolamine 10^{-6} M present in all records. Note the emergence of a small 'fast' depolarization (f) after propranolol. Room temperature.

adrenoceptor-mediated component in this response (cf. Figure 2a and c). On one occasion using iontophoresis of noradrenaline (see later), some responses mediated by α-receptor activation were detected. These were rare, indicating that the contribution to the depolarizations by α-receptor activation was small, thus, subsequently phentolamine (10^{-6} M) was routinely included in the bathing solution to ensure that the major components of the overall depolarization were uncontaminated by the small α-receptor-mediated contribution (if present).

The 'fast' and 'slow' components of depolarization to noradrenaline were dose-dependent and maximum amplitudes of 17 mV and 22 mV respectively were recorded. Propranolol (10^{-7}–5×10^{-7}M) reduced or abolished the 'slow ' depolarization (Figure 2e and f) suggesting its mediation via β-adrenoceptors. This result is surprising since β-receptor-mediated actions in smooth muscle are unusually inhibitory. In contrast, the amplitude of the 'fast' depolarization was not antagonized but rather, on occasions, enhanced in the presence of propranolol (Figure 2e and f) and thus represents the action of noradrenaline on a population of receptors that are neither α- or β-adrenoceptors and are presumably γ-adrenoceptors (neither component of the depolarization was antagonized by methysergide 5×10^{-6} M). An interesting observation was that both phentolamine and propranolol themselves produced depolarization on some occasions. These responses either declined or persisted but these experiments in which the antagonists displayed agonist activity were not used to investigate the pharmacological nature of the receptors activated by noradrenaline or isoprenaline. Presumably phentolamine and propranolol possess agonist activity in the basilar vessels of neonatal rats which is a finding that warrants further investigation.

It should be noted that in 2 experiments the 'fast' γ-adrenoceptor mediated response was elicited when noradrenaline was applied by bath perfusion. On both of these occasions the γ-receptor depolarization was evoked by 5×10^{-7}M noradrenaline. The propranolol-sensitive 'slow' depolarization was readily obtained with bath perfusion of noradrenaline and the threshold concentration was about 10^{-7}M.

The presence of a possible excitatory β-adrenoceptor-mediated response to noradrenaline prompted an investigation of the effects of isoprenaline, a selective β-adrenoceptor agonist. Isoprenaline (2–50 nmol) evoked dose-dependent 'slow' depolarizations which peaked within 30 to 60 s and were often preceded by small (1–2 mV) hyperpolarizations (Figure 3a–e). Both responses, the hyperpolarization and the 'slow' depolarization, were abolished by propranolol (10^{-7}–10^{-6}M, Figure 3e and f) suggesting the presence of classical inhibitory β-receptors and excitatory β-receptors. In the presence of propranolol, isoprenaline often evoked a 'fast' depolarization (Figure 3f) of similar time course to that produced by noradrenaline and therefore presumably mediated by

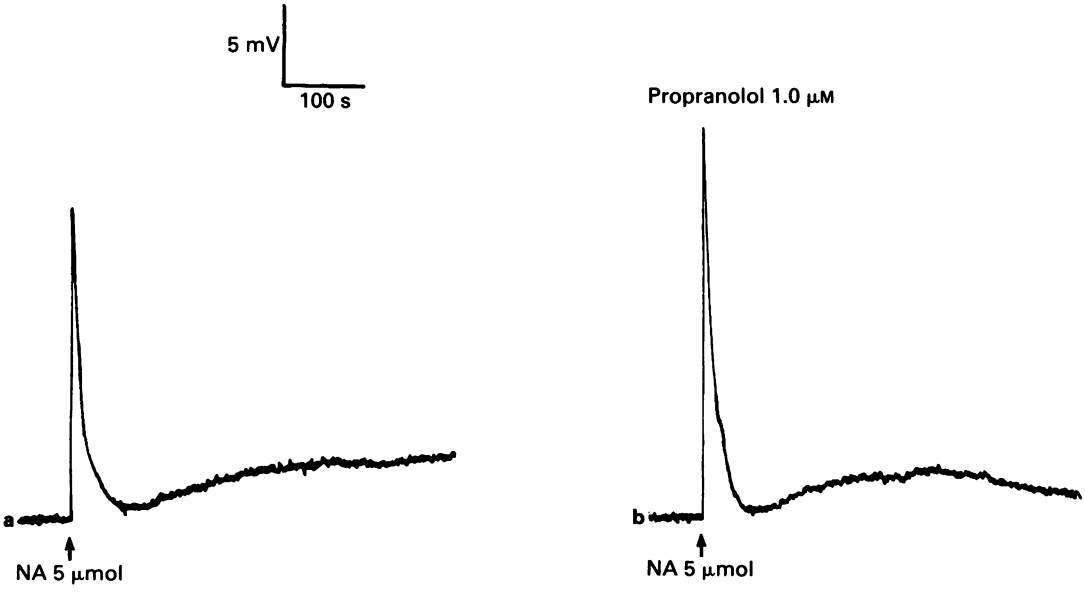

Figure 4 Effects of propranolol (10^{-6} M) on the depolarization to noradrenaline (NA) in the mature (6 weeks old) rat basilar artery. Records (a) and (b) are from the same cell ($E_m = -55$ mV) in the presence of phentolamine (10^{-6} M). Room temperature.

γ-adrenoceptors. Blockade by propranolol of the 'slow' depolarization could be overcome by increasing the dose of isoprenaline but on occasion it appeared that the maximum response was reduced. Because of the difficulty of carrying out repeated dose-response curves in single cells these data were not confirmed. Bath perfusion of isoprenaline (threshold concentration of 10^{-8} M) always produced a slow depolarization in vessels taken from neonatal rats.

Experiments on the basilar artery of mature rats (6 weeks old)

The micro-injection of noradrenaline (1–5 μmol) onto the basilar artery of a 6 week old rat evoked a large 'fast' depolarization (as much as 20 mV was observed) and was followed by a small slow depolarization. Neither of these responses was antagonized by phentolamine or propranolol (Figure 4). Thus in mature rats the basilar artery appears to possess the 'fast' γ-receptor-mediated response but the excitatory β-receptor has been largely eliminated. Presumably the 'slow' depolarization may be mediated by a slow γ-receptor and may correspond to the slow tail of the e.j.p. (see Figure 7) but we did not investigate this response further.

Ionophoresis of noradrenaline and isoprenaline

A series of experiments were carried out in which agonists were applied by ionophoresis to investigate whether the various types of adrenoceptors are distributed uniformly over the muscle surface of neonatal rat basilar arteries or whether receptors are clustered as might occur as a forerunner of synapse formation. Since in the earlier experiments it was demonstrated that the time course of the responses evoked by γ- and excitatory β-receptors are different when the agonists are injected into the organ bath it seemed that the application by ionophoresis of agonists would be suitable for this study because of the superior temporal and spatial resolution of this method of drug application.

In rats 2–21 days old, the ionophoretic application of noradrenaline produced charge-dependent depolarizations on most of the occasions tested (Figure 5a and b). To obtain these responses the amount of noradrenaline that had to be ejected was large compared to previous studies in the anococcygeus muscle where adrenoceptor responses in response to ionophoretic application of noradrenaline have been studied (Large, 1982; 1983). Thus for example, the depolarization shown in Figure 5a has an amplitude of

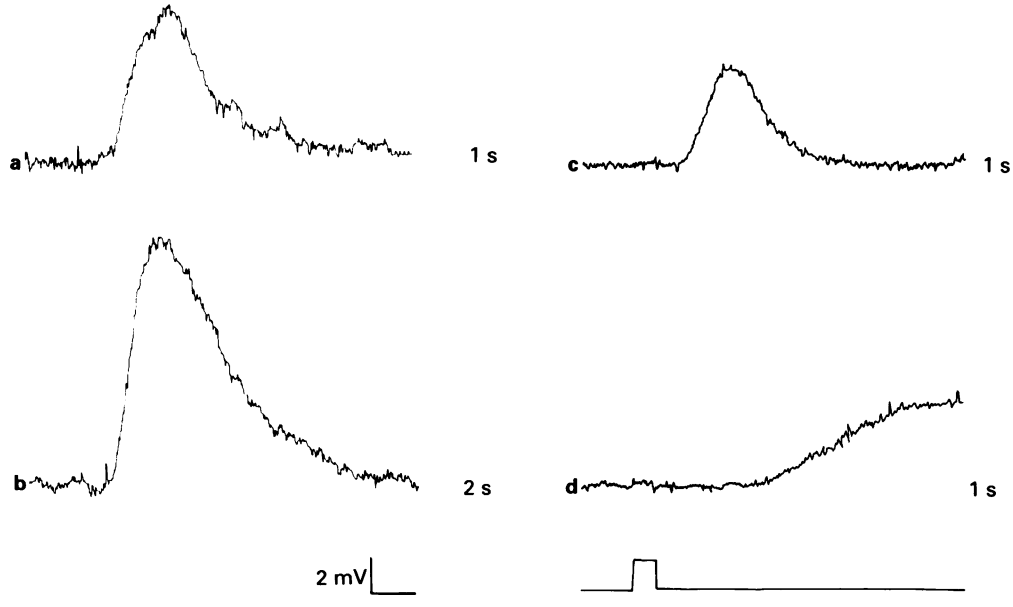

Figure 5 Membrane responses evoked by the ionophoretic application of noradrenaline in the developing rat basilar artery: (a) and (b) were recorded from a 15 day old rat, $E_m = -58$ mV, temperature: 33°C; (c) was recorded from the cell of 14 day old rat, $E_m = -49$ mV, temperature: 22°C and (d) was recorded from a 16 day old rat, $E_m = -69$ mV, temperature: 33°C. Noradrenaline was applied with an ionophoretic pulse of 50 nA in amplitude for the durations shown at the right of the records. Horizontal calibration, 10 s for (a) and (b) and 2 s for (c) and (d). The lower record in (d) is a monitor of the voltage applied to the tip of the ionophoretic electrode.

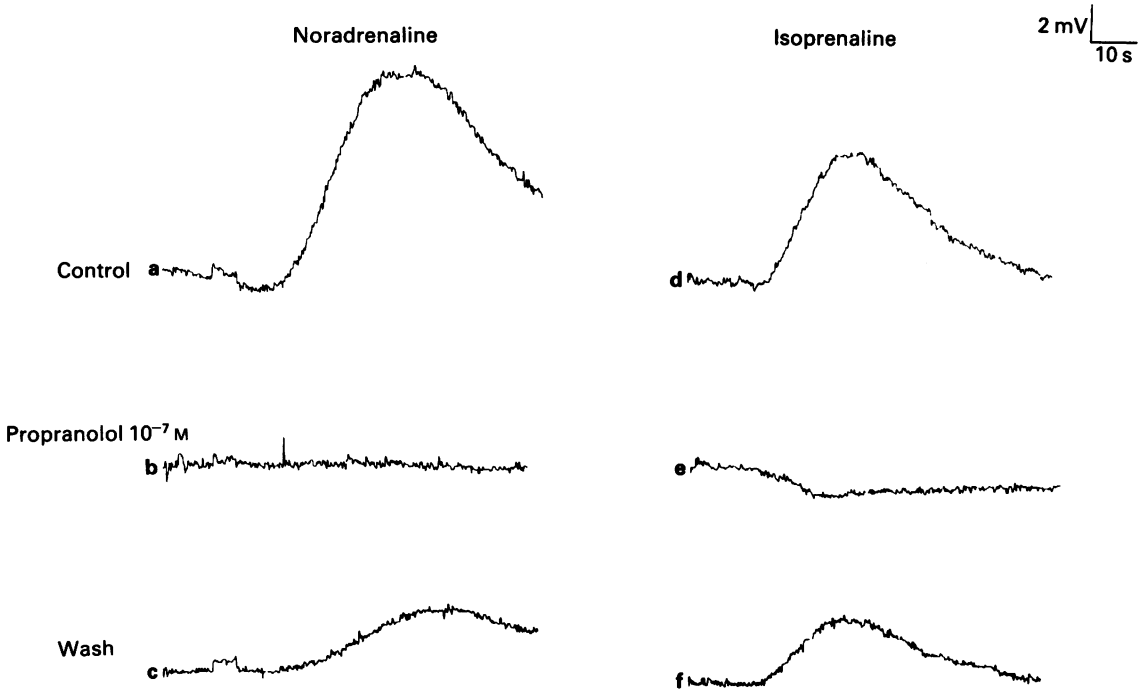

Figure 6 The effect of propranolol on the depolarizations produced by the ionophoretic application of noradrenaline and isoprenaline: (a)–(c) were recorded from 1 cell in a 7 day old rat ($E_m = -69$ mV) and (d)–(f) were recorded from a 21 day old rat ($E_m = -68$ mV). In all records the ionophoretic pulse was 50 nA in amplitude and 0.5 s in duration. Phentolamine 10^{-6} M was present throughout and the temperature was 20°C. (c) and (f) were recorded 85 and 70 min respectively after washing out the propranolol.

9 mV, 50 nC charge was passed by the noradrenaline ionophoretic pipette and therefore the sensitivity of the tissue to noradrenaline was 0.18 mV nC^{-1}. In the anococcygeus muscle, where the noradrenaline-induced depolarizations are mediated by α-adrenoceptors, the sensitivity is usually within the range of 20–50 mV nC^{-1}. Thus the sensitivity of the neonatal rat basilar artery is only about one hundredth of that of the anococcygeus muscle. A possible explanation for this difference in sensitivity to ionophoretically applied noradrenaline is that the density of excitatory β-receptors (see below) in the basilar artery which mediate the depolarization in this tissue is considerably less than the density of the α-adrenoceptors in the anococcygeus muscle. Other possibilities include a low affinity of noradrenaline for the excitatory β-receptor, or if the depolarization is mediated by opening of ion channels, the channel conductance may be low or that the probability of channel opening is low.

The amplitude of most of these depolarizations (e.g. those in Figure 5a and b) was similar whether or not phentolamine (10^{-6} M) was present in the bathing

Table 1 Time course of depolarizations evoked by ionophoretic application of noradrenaline and isoprenaline

	Latency (s)	*Time to peak* (s)	*Latency* (s)	*Time to peak* (s)
Noradrenaline (β-excitatory)	20.0 ± 0.1 (20–23°C) ($n = 20$)	52.0 ± 1.9	6.0 ± 0.3 (33–34°C) ($n = 13$)	18.4 ± 1.2
Noradrenaline (α-excitatory)	1.8 ± 0.1 ($n = 7$)	4.3 ± 0.3	—	
Isoprenaline (β-excitatory)	—		6.8 ± 0.4 ($n = 17$)	26.5 ± 1.7

solution so it seemed that they were not mediated by α-receptors and indeed these responses were blocked by 10^{-7} M propranolol (Figure 6a–c) and so are mediated by the excitatory β-receptor described in the earlier section. Figure 6 also illustrates that the action of propranolol was slow to reverse; even after 70 min washing in propranolol-free solution it was not possible to obtain responses of control amplitude.

It seems that in some neonatal basilar arteries there are α-adrenoceptors, as in one muscle of a 14 day old rat it was possible to record a depolarization (Figure 5c) which was subsequently blocked by phentolamine. In this tissue it was possible to record these α-receptor-mediated depolarizations in all 7 cells impaled and an interesting observation was that in this particular muscle no convincing β-excitatory responses were obtained. The α-receptor-mediated depolarization (Figure 5c) had a more rapid time course than the β-receptor-mediated response (Figure 5d). At 33–34°C the latency (time between beginning of ionophoretic pulse and onset of depolarization) of the β response was 6.0 s and the total time to peak was 18.4 s (Table 1). The time course of the β response was temperature-sensitive and cooling the preparations to room temperature (20–23°C) increased the latency and time to peak to 20.0 and 52.0 s respectively (Table 1). In contrast the depolarizations mediated by α-receptor activation (e.g Figure 5c) had a faster time course and even at room temperature the latency was only 1.80 s and time to peak, 4.30 s (Table 1). This difference in time course between the α- and the excitatory β-depolarizations is shown in Figure 5c and d in which the same amount of noradrenaline was ionophoresed to obtain both responses which are shown on the same time base; it should be noted that the comparison is highlighted by the fact that the α-response was recorded at 22°C while the β-depolarization was recorded at 33°C (at room temperature the β-response would not even have started using the time calibration in Figure 5c and d).

The ionophoretic application of isoprenaline also depolarized the cells in neonatal basilar artery with a similar time course to the noradrenaline β-receptor mediated depolarizations (Figure 6d and f and Table 1); these responses were blocked by propranolol (Figure 6e). It can be seen from Figure 6e that isoprenaline elicited a small hyperpolarization which, although recorded on other occasions in the presence of propranolol and phentolamine, was inconsistent and presumably does not result from either α- or β-receptor activation. We have no explanation for this response and it was not investigated further.

There are two important points to make from the ionophoretic experiments. Firstly, the excitatory β-receptors appeared to be distributed fairly evenly over the muscle surface. There were a few occasions when noradrenaline or isoprenaline did not evoke a response but the ionophoretic charges used were large and the electrodes quite frequently blocked during passage of current in that the voltage required to pass current through the electrode tip exceeded the capability of the current pump. Thus on those occasions when no response was obtained it is possible that the ionophoretic electrode failed to expel the drug. Thus clustering of excitatory β-receptors in neonatal basilar artery cannot be discounted but there were many occasions when repeated placement of the inophoretic electrode tip at 50–100 μm intervals produced depolarizations of a similar amplitude and so in these tissues at least, the excitatory β-receptors were fairly evenly distributed over the smooth muscle.

Secondly, there was no consistent sign of a fast response which could be attributed to γ-adrenoceptor activation in any of the tissues investigated (cf. Hirst & Neild, 1980a), even in mature basilar vessels in which a phentolamine-resistant e.j.p. could be recorded (illustrating the formation of synapses) and thus these blood vessels may be different from the submucous mesenteric arterioles of the guinea-pig.

The results from the ionophoretic experiments also confirmed the data from the experiments in which drugs were injected into the organ bath as in mature rats (6 weeks old) neither noradrenaline or isoprenaline produced an excitatory response that was sensitive to propranolol. Moreover, neither component of

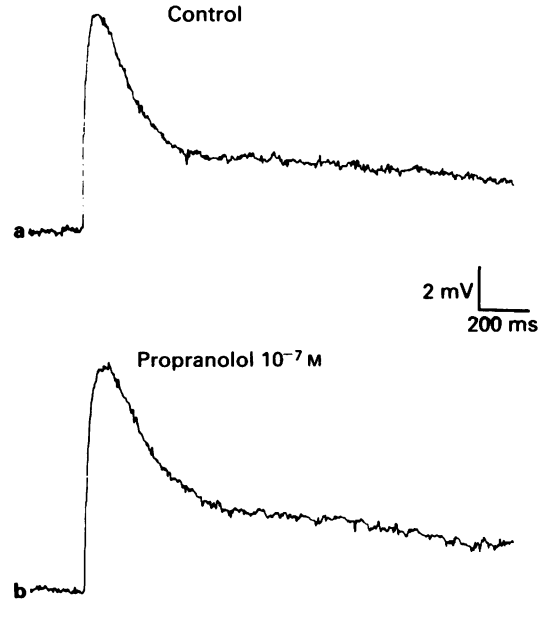

Figure 7 The lack of effect of propranolol on the biphasic e.j.p. recorded from a 21 day old rat basilar artery. $E_m = -57$ mV, temperature: 22°C and phentolamine (10^{-6} M) was present throughout.

the biphasic e.j.p. in mature basilar vessels was antagonized by propranolol (Figure 7) and these data taken together suggest that the excitatory β-adrenoceptor had largely regressed in basilar vessels from mature rats and does not contribute to the synaptic electrical response.

Discussion

The main finding of the present paper is that the electrical responses of the basilar artery from neonatal rats are mediated largely by excitatory β- and γ-adrenoceptors. During maturation the excitatory β-receptors regress and the γ-receptors are predominant. This is in agreement with the data of Hirst *et al.* (1982) who found in basilar arteries from adult rats that the depolarizations produced by bath-applied noradrenaline were unaffected by phentolamine and propranolol. There appears to be only a very few α-receptors in neonatal basilar vessels as phentolamine-resistant depolarizations could be recorded in only a few vessels and usually when observed, the contribution of the α-response was small compared to the depolarizations evoked by γ- and β-receptor stimulation (e.g. Figure 2). Some evidence for the existence of inhibitory β-adrenoceptors in neonatal basilar arteries was found; the depolarizations elicited by isoprenaline were usually preceded by small transient hyperpolarizations which were blocked by propranolol (Figure 3). Moreover in preparations from neonates, propranolol often enhanced the fast γ-response to noradrenaline (Figure 2) and even unmasked a fast γ-response to isoprenaline (e.g. Figure 3). In basilar arteries from mature rats there may be some inhibitory β-receptors as the γ-response to noradrenaline was slightly potentiated by propranolol but this effect was very small. In cerebral arteries from other animal species, there appear to be inhibitory β-receptors in the adult cat (Edvinsson & Owman, 1974; Harder *et al.*, 1981) but they are absent in the rabbit (Duckles & Bevan, 1976). However, the striking species difference is the lack of α-adrenoceptors in the adult rat basilar, confirming the report of Hirst *et al.* (1982). In contrast, α-receptors are present in adult cat and rabbit cerebral vessels (Edvinsson & Owman, 1974; Duckles & Bevan, 1976; Harder *et al.*, 1981). The term γ-adrenoceptor was not used in these papers but Duckles & Bevan (1976) found that in the rabbit basilar artery the noradrenaline concentration-effect (increase in tension) curve consisted of two components associated with low and high concentrations of noradrenaline. The equilibrium dissociation constants for phentolamine against these two components were about 5×10^{-8} M and 2×10^{-6} M respectively. The simplest explanation for these data in the rabbit is that low concentrations of noradrenaline act on α-adrenoceptors while high concentrations stimulate the γ-receptor.

The excitatory β-response observed in these experiments is intriguing as there are so few reports of β-excitation in smooth muscle. Edvinsson & Owman (1974) demonstrated that isoprenaline contracted the cat middle cerebral artery and that it was antagonized by low concentrations of propranolol but these authors argued that the response to isoprenaline was not mediated by β-receptors as both the slope and the maximum response of the isoprenaline dose effect were depressed by propranolol. Although we found evidence for this latter characteristic in our experiments, we chose to use the term β-excitation for the slow depolarization in neonatal basilar vessels because of the order of potency of agonists (isoprenaline > noradrenaline) and because low concentrations of propranolol (10^{-7} M, which is no more than ten times the equilibrium dissociation constant in other tissues) abolished submaximal depolarizations. Thus although the non-competitive characteristics of propranolol antagonism indicate that this receptor is not the same as the β-receptor say in cardiac tissue, for the sake of simplicity it was decided to use the notation of excitatory β-receptor. One characteristic of this β-depolarization was the very slow kinetics, at room temperature the latency of the depolarization (produced by ionophoresis of noradrenaline) being 20 s. Presumably at the time of the onset of depolarization the free concentration of agonist would be negligible and thus the slow kinetics are a characteristic of the response. In cases where the time course of depolarizations are very temperature-sensitive (in this experiment the latency of the β excitatory response has a Q_{10} of about 3) the usual explanation is that some biochemical reaction takes place between drug-receptor binding and onset of response.

The demonstration that the fast depolarization evoked by noradrenaline in both neonatal and adult basilar arteries is resistant to phentolamine and propranolol is further evidence for the existence of the γ-adrenoceptor. Generally rather high doses (0.1 – 1 μmol) of noradrenaline were necessary to evoke the γ-receptor-mediated depolarization but responses were obtained to 10 nmol noradrenaline and in this situation the maximum concentration is unlikely to exceed 40 μM and in some bath-perfusion experiments the γ-receptor mediated response was elicited by 5×10^{-7} M noradrenaline. Our impression is that the method of application of noradrenaline is critical because of γ-receptor desensitization, as occurs between noradrenaline and the junctional α-adrenoceptor in the anococcygeus muscle (Large, 1983). The question of the high concentrations of noradrenaline necessary to activate the γ-adrenoceptor has been discussed by Hirst *et al.* (1982) and presumably suggests that the affinity of noradrenaline for the

γ-adrenoceptor is very low as is the case for acetylcholine and the nicotinic receptor and glutamate for its receptor. It is possible that the added noradrenaline releases some other substance which depolarizes the arterial smooth muscle and accounts for the response. However the rapid response (sometimes 300 ms) argues against such an indirect effect. The two most likely sources of release of a secondary chemical in this isolated preparation are nerve terminals and endothelium. The former possibility is unlikely as the sympathetic innervation is not fully developed in neonatal rats (see Hill *et al.*, 1983) and field stimulation (100 V, 0.2 ms pulse width) did not evoke a perceptible response in the present experiments. Also we feel that little of the noradrenaline applied by brief injection could reach the endothelium which is situated within the lumen of the blood vessel. Another explanation is that the 'fast' depolarization produced by noradrenaline is not the consequence of activation of a γ-adrenoceptor but rather is caused by a non-specific action of noradrenaline on another type of pharmacological receptor. In this case one of the most likely candidates is the 5-hydroxytryptamine (5-HT) receptor but methysergide had no effect on submaximal γ-receptor depolarizations and also muscarinic and nicotinic antagonists were without effect. Injection of adenosine triphosphate (ATP) onto the basilar artery also produces a rapid depolarization (unpublished data) but we have some preliminary evidence that bath perfusion of ATP desensitizes the basilar artery to injected ATP whereas injected noradrenaline still produces a rapid depolarization in the presence of ATP. Therefore it is unlikely that the 'fast' depolarization is caused by a non-specific action of noradrenaline but rather by activation of the γ-adrenoceptor.

The question arises whether this receptor mediates the e.j.p. in blood vessels as has been suggested previously (Hirst & Neild, 1980a and b). This problem is of obvious importance especially in the light of the recent suggestion that the e.j.p. in the guinea-pig vas deferens is produced by the action of ATP (Sneddon & Westfall, 1984). Also recently it has been shown that the e.j.p. produced by activation of the α_1-adrenoceptor in the rat anococcygeus muscle is sometimes preceded by a more rapid e.j.p. (Byrne & Large, 1984). The similarity of the time course of this rapid e.j.p. and the depolarization produced by ionophoretic application of ATP led to the suggestion that this rapid e.j.p. in the anococcygeus is caused by ATP. With regard to the present experiments it is interesting to note that the time to peak of the e.j.p. in the rat basilar artery at room temperature is about 100 ms and a similar value is found in the rat tail artery (unpublished data). This value is similar to values found at 37°C for various muscular arterioles (e.g. Surprenant, 1980) but is significantly shorter than the time to peak of the fast e.j.p. and depolarization produced by ATP in the rat anococcygeus muscle at room temperature (both values are about 300 ms, Table 1, Byrne & Large, 1984). This evidence tends to argue against the role of ATP in producing the e.j.p. in blood vessels and by implication supports the physiological function of the γ-adrenoceptor in sympathetic neurotransmission.

We were surprised that it was not possible to obtain fast γ-adrenoceptor responses using ionophoresis of noradrenaline even when ejecting large amounts onto tissues which responded with γ-receptor-mediated depolarizations on micro-injection of noradrenaline. The failure to observe γ-receptor-mediated depolarizations with ionophoresis may be related to the technical limitation concerning the amount of noradrenaline that can be ejected using this technique, a problem which would be exacerbated by the low affinity of noradrenaline for the γ-receptor as suggested earlier. Also it is possible that γ-receptors occur in small clusters separated by regions of muscle membrane which possess few or no γ-receptors. If each cluster contained only a few receptors then the localized application of noradrenaline by ionophoresis might not activate a sufficient number of receptors to elicit a perceptible response and an observable depolarization would be obtained only when a large number of receptors are stimulated as probably occurs when a micro-injection is used. An alternative explanation is that the γ-receptors are uniformly distributed over the muscle but in a low concentration. From ionophoretic experiments it appears that the excitatory β-receptors are uniformly distributed and so it would be necessary to postulate that the density of γ-receptors, if uniformly distributed, must be considerably lower than the β-receptor density. However in experiments where noradrenaline was added by micro-injection the amplitudes of the γ- and β-responses were similar (e.g. Figure 2) and so it is possible that γ-receptors are clustered into small groups, as might occur for example if they mediated the synaptic response. Of course the syncytical nature of the smooth muscle tissue would ensure the maximum effectiveness of these clusters when many synaptic regions are activated simultaneously as might occur during nerve stimulation.

This work was supported by the Medical Research Council.

References

BYRNE, N.G. & LARGE, W.A. (1984). Comparison of the biphasic excitatory junction potential with membrane responses to adenosine triphosphate and noradrenaline in the rat anococcygeus muscle. *Br. J. Pharmac.*, **83,** 751–758.

BYRNE, N.G. & MUIR, T.C. (1985). Electrical and mechanical responses of the bovine retractor penis to nerve stimulation and to drugs. *J. Auton. Pharmac.*, **4,** 261–271.

BYRNE, N.G., HIRST, G.D.S. & LARGE, W.A. (1985). The nature of the adrenoceptors in the isolated rat basilar artery during development. *J. Physiol.*, (in press).

DUCKLES, S.P. & BEVAN, J.A. (1976). Pharmacological characterization of adrenergic receptors of a rabbit cerebral artery in vitro. *J. Pharmac. exp. Ther.*, **197,** 371–378.

EDVINSSON, L. & OWMAN, C. (1974). Pharmacological characterization of adrenergic alpha and beta receptors mediating the vasomotor responses of cerebral arteries in vitro. *Circulation Res.*, **35,** 835–849.

FOLKOW, B. & NEIL, E. (1971). *Circulation.* Oxford: Oxford University Press.

HARDER, D.R., ABEL, P.W. & HERMSMEYER, K. (1981). Membrane electrical mechanism of basilar artery constriction and pial artery dilation by norepinephrine. *Circulation Res.*, **49,** 1237–1242.

HILL, C.E., HIRST, G.D.S. & VAN HELDEN. D.F. (1983). Development of sympathetic innervation to proximal and distal arteries of the rat mesentery. *J. Physiol.*, **338,** 129–147.

HIRST, G.D.S. & NEILD, T.O. (1980a). Evidence for two populations of excitatory receptors for noradrenaline on arteriolar smooth muscle. *Nature*, **283,** 767–768.

HIRST, G.D.S. & NEILD, T.O. (1980b). (Matters arising): Noradrenergic transmission: Reply to McGrath. *Nature*, **288,** 301–302.

HIRST, G.D.S., NEILD, T.O. & SILVERBERG, G.D. (1982). Noradrenaline receptors on the rat basilar artery. *J. Physiol.*, **328,** 351–360.

LARGE, W.A. (1982). Membrane potential responses of the mouse anococcygeus muscle to ionophoretically applied noradrenaline. *J. Physiol.*, **326,** 385–400.

LARGE, W.A. (1983). Membrane potential responses to ionophoretically applied α-adrenoceptor agonists in the mouse anococcygeus muscle. *Br. J. Pharmac.*, **79,** 233–243.

NORBERG, K.A. & HAMBERGER, B. (1964). The sympathetic neuron. Some characteristics revealed by histochemical studies on the intraneuronal distribution of transmitter. *Acta physiol. .scand.*, Suppl. **238,** 1–42.

PURVES, R.D. (1979). The physics of iontophoretic pipettes. *J. Neurosci. Methods.*, **1,** 165–178.

SURPRENANT, A. (1980). A comparative study of neuromuscular transmission in several mammalian muscular arteries. *Pflügers Arch. ges. Physiol.*, **386,** 85–91.

SNEDDON, P. & WESTFALL, D.P. (1984). Pharmacological evidence that adenosine triphosphate and noradrenaline are co-transmitters in the guinea-pig vas deferens. *J. Physiol.*, **347,** 561–580.

(Received February 27, 1985.
Revised April 15, 1985.
Accepted May 8, 1985.)