Br. J. Pharmac. (1977), **59**, 635–641

THE PHARMACOLOGY OF ADRENERGIC NEURONAL RESPONSES IN THE CEREBRAL CORTEX: EVIDENCE FOR EXCITATORY α- AND INHIBITORY β-RECEPTORS

P. BEVAN, C.M. BRADSHAW & E. SZABADI

Department of Psychiatry, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT

1 The technique of microelectrophoresis was used to compare the actions of a range of adrenoceptor agonists on single cortical neurones in the rat anaesthetized with halothane.

2 Phenylephrine and methoxamine were exclusively excitatory, whereas salbutamol was entirely depressant. Noradrenaline and isoprenaline could evoke both excitatory and depressant responses. Lower doses of isoprenaline usually evoked depressions, whereas higher doses, on the same cell, evoked excitatory responses.

3 The α-adrenoceptor blocking agents, phentolamine and phenoxybenzamine, reversibly antagonized excitatory responses to adrenoceptor agonists, without affecting depressant responses to adrenoceptor agonists or excitatory responses to acetylcholine.

4 The β-adrenoceptor blocking agents, propranolol and sotalol, reversibly antagonized both depressant and excitatory responses to adrenoceptor agonists, without affecting responses to acetylcholine. When the effect of sotalol on excitatory and depressant responses to adrenoceptor agonists was compared on the same cell, the depressant responses could be selectively antagonized, without affecting the excitatory responses.

5 It is concluded that (a) responses of cortical neurones to adrenoceptor agonists are mediated by both α- and β-receptors; (b) these α- and β-receptors give rise to opposite effects: the α-receptors being excitatory and the β-receptors being inhibitory; and (c) responses of many neurones reflect the presence of both types of receptor.

Introduction

Single cortical neurones are sensitive to noradrenaline (NA) applied by microelectrophoresis: both excitatory and depressant responses have been described (Krnjević & Phillis, 1963; Johnson, Roberts, Sobieszek & Straughan, 1969; Bevan, Bradshaw, Roberts & Szabadi, 1974a). So far, however, these responses have not been characterized in terms of the classical α- and β-adrenoceptors (Ahlquist, 1948).

There are basically three methods available for receptor categorization: comparison of agonists; comparison of antagonists; and desensitization (Schild, 1973). Previous attempts to classify neuronal responses concentrated mainly on the use of antagonists. It has been reported that both α- and β-receptor blocking agents can antagonize excitatory responses to NA whereas depressant responses to NA seem to be much more resistant to these antagonists (Johnson *et al.*, 1969). There are fewer data concerning the effects of different adrenoceptor agonists. Although it has been reported that isoprenaline (IPNA) can evoke both excitatory and depressant responses on cortical neurones (Johnson *et al.*, 1969), the effects of more selective β-adrenoceptor stimulants (e.g. salbutamol), or the effects of more selective α-receptor stimulants (e.g. phenylephrine, methoxamine) have never been tested.

In the experiments presented here we have compared the effects of a range of α- and β-receptor stimulating agents on cortical neurones. We have also re-examined the selectivity of adrenoceptor antagonists. Our results strongly suggest that the excitatory responses of cortical neurones to NA are mediated by α- whereas the depressant responses are mediated by β-receptors.

Some of these results have been presented to the British Pharmacological Society (Bevan, Bradshaw & Szabadi, 1976b).

Methods

Adult male albino rats (250–300 g) were used. The animals were anaesthetized with halothane (0.8–1.0%). Our methods for the surgical preparation

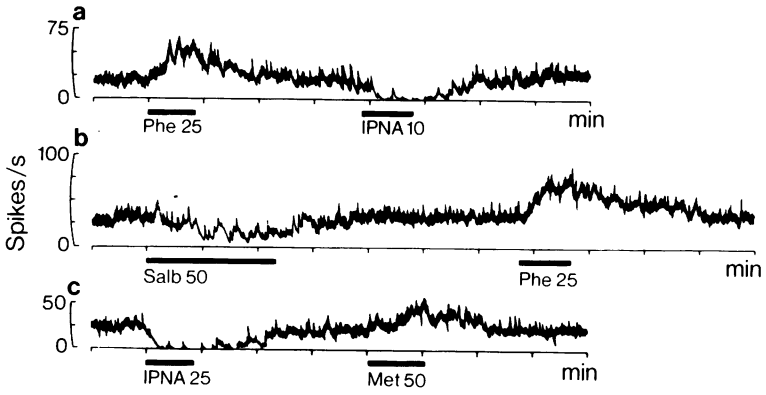

Figure 1 Examples of the effects of adrenoceptor agonists on cortical neurones. Ratemeter recordings of the firing rates of three cortical neurones in the rat (a, b, c). Ordinate scale: firing rate (spikes/s); abscissa scale: running time (min). Horizontal bars indicate microelectrophoretic drug applications; numbers refer to intensities of ejecting currents (nA). (a) A cell excited by phenylephrine (Phe) and depressed by isoprenaline (IPNA); (b) a cell depressed by salbutamol (Salb) and excited by phenylephrine; (c) a cell depressed by isoprenaline and excited by methoxamine (Met).

of the animals and the manufacture of six-barrelled glass micropipettes for extracellular recording and microelectrophoretic drug application, have been described previously (Bradshaw, Roberts & Szabadi, 1973a; Bradshaw, Szabadi & Roberts, 1973b; Bevan, Bradshaw & Szabadi, 1976a).

Micropipettes having tip diameters of 3–5 μm were used. Two barrels of each micropipette contained 4 M NaCl, one for recording action potentials, the other for use in current balancing. The remaining barrels contained drug solutions. The following drug solutions were used: (−)-noradrenaline bitartrate (0.05 M, pH 3.0–3.5); (−)-phenylephrine hydrochloride (0.05 M, pH 5.0–5.5); (±)-methoxamine hydrochloride (0.05 M, pH 4.5); salbutamol sulphate (0.05 M, pH 5.5); (±)-isoprenaline hydrochloride (0.05 M, pH 5.5); acetylcholine chloride (0.05 M, pH 3.5–4.0); phentolamine mesylate (0.01 M, pH 4.5–5.5); phenoxybenzamine hydrochloride (0.01 M, pH 3.0); propranolol hydrochloride (0.01 M, pH 4.5–5.5); sotalol hydrochloride (0.01 M, pH 4.0–5.0).

Only spontaneously active neurones were studied in these experiments. All the drugs were applied by microelectrophoresis. When a suitable unit was encountered the agonists were applied in a regular cycle. Between successive drug applications retaining currents of −10 nA were passed. Retaining currents of −25 nA were used for the antagonists. Intervals between successive applications of the same agonist were kept constant in order to standardize the effects of the retaining current upon drug release during the ejection period (Bradshaw *et al.*, 1973a,b). The sizes of the neuronal responses to the agonists were expressed as the total number of action potentials produced in response to each drug application ('total spike number', Bradshaw *et al.*, 1973b).

The effects of antagonists were evaluated in the following way. When suitable responses to the agonists had been obtained, the antagonist was applied continuously, either by removal of the retaining current (thus allowing the drug to diffuse out from the micropipette), or by the passage of a weak ejecting current (5–10 nA), and the time course of the developing antagonism was followed. If necessary the intensity of the ejecting current was increased until antagonism was observed. After antagonism of the response to the agonist had been observed, application of the antagonist was continued until a further response to the control agonist(s) could be observed. Then the application of the antagonist was terminated and the time course of recovery was followed. The response to an agonist was regarded as antagonized if there was at least a 50% reduction in the total spike number (Bevan *et al.*, 1974a).

Results

Effects of adrenoceptor agonists

The proportion of cells responding with excitation (increase in firing rate) or depression (decrease in firing rate) to each adrenoceptor agonist studied are shown in Table 1. It is apparent from the table that phenylephrine and methoxamine were exclusively excitatory, whereas salbutamol was entirely depressant. On the other hand, NA and IPNA could evoke both excitatory and depressant responses. Examples of cells responding both with excitation and depression to different agonists are shown in Figure 1.

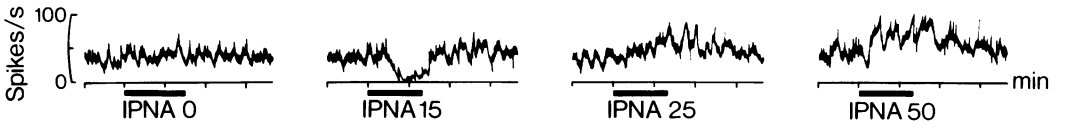

Figure 2 Responses of a cortical neurone to isoprenaline (IPNA) applied with increasing intensities of ejecting current. Ratemeter recording of firing rate of a single cortical neurone (as in Figure 1). Note the reversal of the response from depression to excitation as the intensity of the ejecting current was increased.

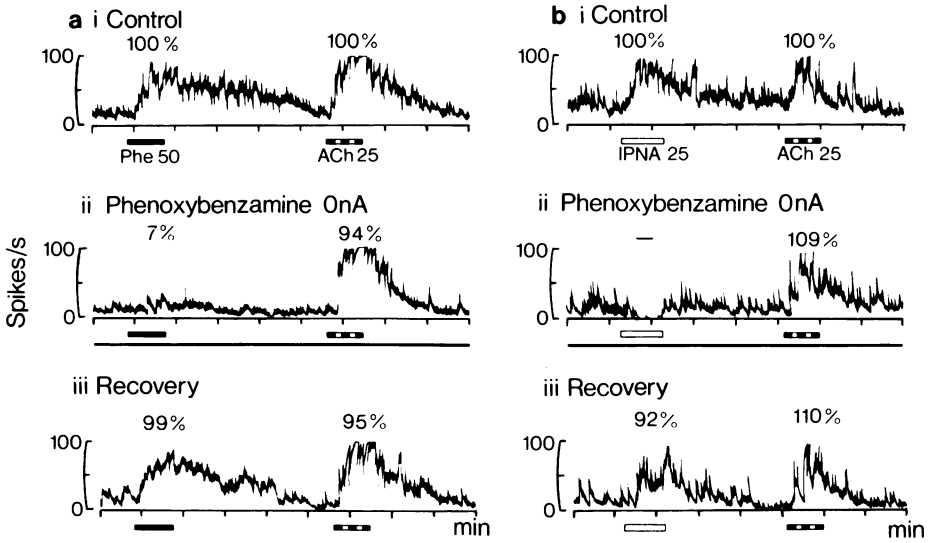

Figure 3 Effects of phenoxybenzamine on excitatory responses to adrenoceptor agonists and acetylcholine. Ratemeter recordings of the firing rates of two cortical neurones (a) and (b) (as in Figure 1). Figures above the traces indicate total spike numbers (%), taking the sizes of the control responses to each agonist as 100%. (a) Effect of phenoxybenzamine on excitatory responses to phenylephrine (Phe) and acetylcholine (ACh): (i) control responses to the agonists; (ii) responses to the agonists during the continuous application of phenoxybenzamine. Phenoxybenzamine was applied by removal of the retaining current (0 nA), and at the start of trace (ii) had been applied continuously for 5 minutes. The responses to phenylephrine, but not the response to ACh, was antagonized. (iii) Recovery of the response to phenylephrine 15 min after the application of phenoxybenzamine had been terminated. (b) Effect of phenoxybenzamine on excitatory responses to isoprenaline (IPNA) and acetylcholine (ACh): (i) control responses to the agonists; (ii) responses to the agonists during the continuous application of phenoxybenzamine. At the start of trace (ii) phenoxybenzamine (0 nA) had been applied continuously for 12 minutes. The excitatory response to IPNA was antagonized, revealing a depressant response, whereas the response to ACh was not affected. (iii) Recovery of the response to IPNA 10 min after the application of phenoxybenzamine had been terminated.

Table 1 Percentage of cortical neurones responding either with excitation or depression to adrenoceptor agonists

	Percentage of cells responding		
	Excitation	*Depression*	(n)
Phenylephrine	100	0	78
Methoxamine	100	0	11
Noradrenaline	66	34	194
Isoprenaline	23	77	138
Salbutamol	0	100	16

IPNA could evoke both depressant and excitatory responses in a dose-dependent fashion on the same cell: on 14 cells a lower current of IPNA evoked a depression, whereas a higher current evoked an excitation. Examples of this observation are shown in Figure 2. On the basis of this observation, we could predictably evoke depressant responses to IPNA by applying the drug with relatively low ejecting currents (<25 nA) (see the antagonism studies below).

The relative potencies of methoxamine and phenylephrine were compared on 10 cells: in order to obtain approximately equivalent responses to the two

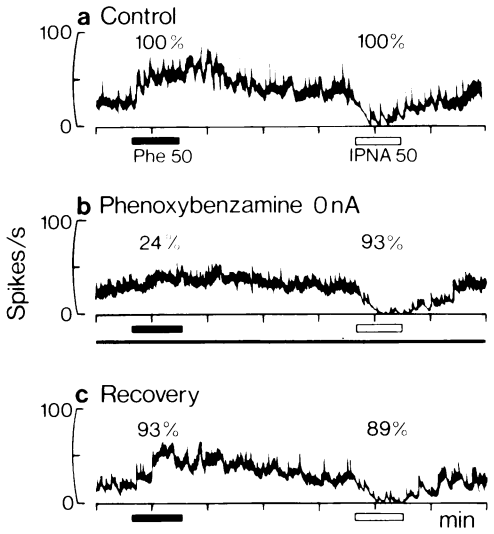

Figure 4 Effects of phenoxybenzamine on excitatory and depressant responses to adrenoceptor agonists. Ratemeter recording of the firing rate of a single cortical neurone (as in Figure 3): (a) control responses to phenylephrine (Phe) and isoprenaline (IPNA); (b) responses to the agonists during the continuous application of phenoxybenzamine. At the start of trace (b) phenoxybenzamine (0 nA) had been applied continuously for 15 minutes. The excitatory response to phenylephrine, but not the depressant response to IPNA, was antagonized. (c) Recovery of the response to phenylephrine 20 min after the application of phenoxybenzamine had been terminated.

drugs, the current needed to apply methoxamine was at least five times greater than that needed to apply phenylephrine.

Effects of adrenoceptor antagonists

α-Adrenoceptor blocking agents The α-adrenoceptor blocking agents, phentolamine and phenoxybenzamine, were effective in reversibly antagonizing excitatory responses to adrenoceptor agonists while excitatory responses to acetylcholine (ACh) were not affected. Phentolamine reversibly and selectively antagonized excitatory responses to NA on 10 cells. Phenoxybenzamine reversibly and selectively antagonized excitatory responses to NA (5 cells), phenylephrine (11 cells), and IPNA (5 cells) (e.g. Figure 3). On one of the cells excited by IPNA, a depressant response was revealed after the abolition of the excitatory response by phenoxybenzamine. On one cell which did not respond to IPNA, a depressant response to IPNA appeared in the presence of phenoxybenzamine; this response disappeared after the application of the antagonist had been terminated.

We have also examined whether the α-adrenoceptor blocking agents can discriminate between excitatory and depressant responses to adrenoceptor agonists on the same cell. In these experiments, excitatory responses were evoked by phenylephrine, depressant responses were evoked by either IPNA or salbutamol, and phenoxybenzamine was used as the antagonist. Antagonism studies were successfully completed on 7 cells; on all these cells the excitatory response was antagonized, whereas the depressant response was not affected. An example of this observation is shown in Figure 4.

β-Adrenoceptor blocking agents The β-adrenoceptor blocking agent, sotalol, was effective in reversibly antagonizing depressant responses to IPNA while responses to ACh were not affected (19 cells) (see Figure 5a). On two cells depressed by IPNA an excitation was revealed after the abolition of the depressant responses by sotalol. On one cell which did not respond to IPNA an excitatory response appeared in the presence of sotalol; this response disappeared after the application of sotalol had been terminated. The β-receptor blocking agents, propranolol and sotalol, could also reversibly antagonize excitatory responses to adrenoceptor agonists, without affecting responses to ACh: excitatory responses to NA were antagonized by propranolol on 4 cells, and excitatory responses to IPNA were antagonized by sotalol on 2 cells (see Figure 5b).

We have also examined whether these antagonists can discriminate between depressant and excitatory responses to adrenoceptor agonists on the same cell. In these experiments depressant responses were evoked by IPNA, excitatory responses were evoked by phenylephrine, and sotalol was used as the antagonist. Antagonism studies were successfully completed on 9 cells. On all these cells, the depressant response was antagonized, whereas the excitatory response was not affected (see Figure 6).

Discussion

The action of adrenoceptor agonists (see Table 1) strongly suggests that the excitatory responses to these drugs are mediated by α-, whereas the depressant responses are mediated by β-receptors. Phenylephrine (Furchgott, 1972; Besse & Furchgott, 1976) and methoxamine (Furchgott, 1970; Innes & Nickerson, 1975) are highly selective α-adrenoceptor agonists, whereas salbutamol is a selective β-receptor stimulant with no affinity for α-adrenoceptors (Brittain, Jack & Ritchie, 1970; Spedding & Weetman, 1972). On the other hand, IPNA and NA can act at both α- and β-receptors (see Furchgott, 1972). It is well documented in the periphery that the dose-response curve to IPNA is a biphasic one: lower

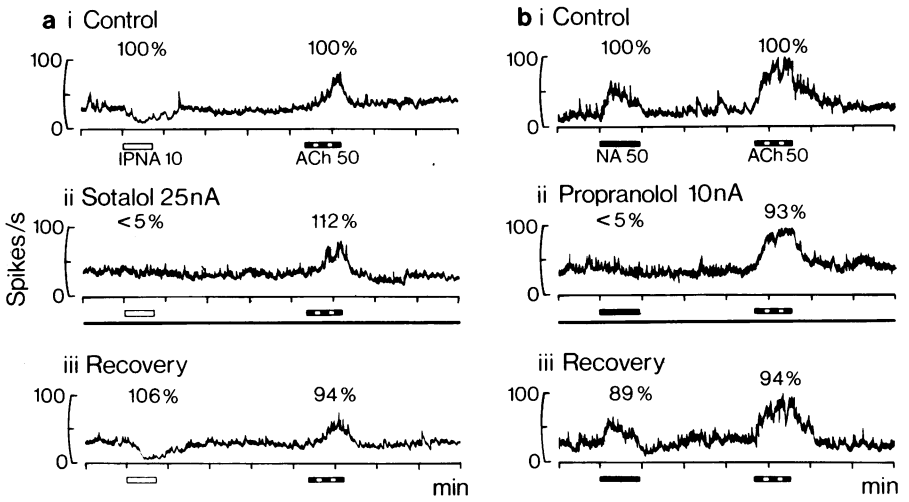

Figure 5 Effects of β-adrenoceptor blocking agents on depressant and excitatory responses to adrenoceptor agonists and excitatory responses to acetylcholine (ACh). Ratemeter recordings of firing rates of two cortical neurones, (a) and (b) (as in Figure 3). (a) Effect of sotalol on depressant response to isoprenaline (IPNA) and excitatory response to ACh: (i) control response to agonists; (ii) responses to agonists during the continuous application of sotalol (25 nA). At the start of trace (ii) sotalol and had been applied continuously for 15 minutes. The depressant response to IPNA, but not the excitatory response to ACh, was antagonized. (iii) Recovery of response to IPNA 13 min after the application of sotalol had been terminated. (b) Effect of propranolol on excitatory responses to noradrenaline (NA) and ACh: (i) control responses to agonists; (ii) responses to agonists during the continuous application of propranolol (10 nA). At the start of trace (ii) propranolol had been applied continuously for 9 minutes. The response to NA, but not the response to ACh, was antagonized. (iii) Recovery of the response to NA 5 min after the application of propranolol had been terminated.

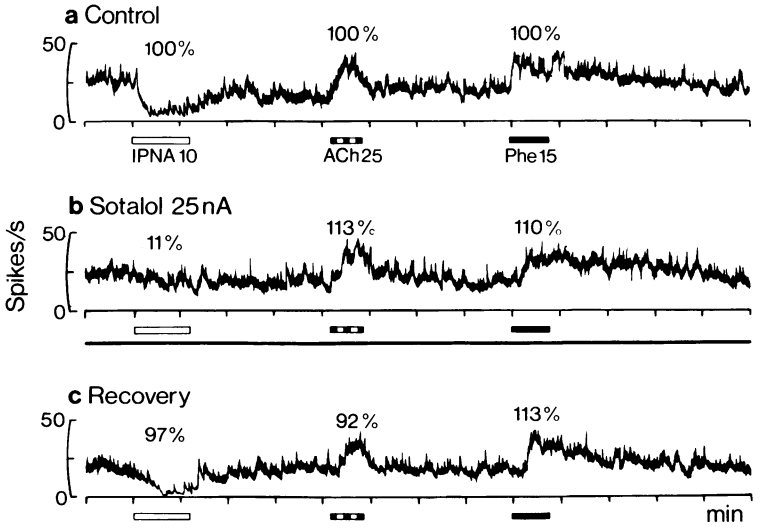

Figure 6 Effects of sotalol on depressant responses to isoprenaline (IPNA) and excitatory responses to phenylephrine (Phe) and acetylcholine (ACh). Ratemeter recording of the firing rate of a single cortical neurone (as in Figure 3): (a) control responses to the agonists; (b) responses to the agonists during the continuous application of sotalol (25 nA). At the start of trace (b) sotalol had been applied continuously for 35 minutes. The depressant response to IPNA, but not the excitatory responses to either ACh or phenylephrine, was antagonized. (c) Recovery of the response to IPNA 35 min after the application of sotalol had been terminated.

doses of the drug relax smooth muscle preparations due to the stimulation of β-receptors, whereas higher concentrations have a contractile effect due to the activation of excitatory α-receptors (Spedding & Weetman, 1972; Trendelenburg, 1974). A very similar observation was made in our experiments: lower doses of IPNA depressed, whereas somewhat higher doses excited the same cortical neurone (see Figure 2).

Methoxamine appeared to be less potent than phenylephrine in our experiments. This is in agreement with observations in the periphery where methoxamine has a considerably lower potency than phenylephrine (Furchott, 1970; Schümann & Endoh, 1976). However, an apparent difference in potency in our experiments might also reflect physical factors, such as difference between the transport numbers and diffusion coefficients of the two drugs (Szabadi & Bradshaw, 1974).

The results with the adrenoceptor antagonists support our suggestion, based on the action of the agonists, that excitatory responses are mediated by α-, and depressant responses are mediated by β-receptors. In agreement with previous findings (Johnson *et al.*, 1969; Bevan *et al.*, 1974a; Bevan *et al.*, 1976a), we have found that the α-adrenoceptor blocking agents could reversibly antagonize excitatory responses to adrenoceptor agonists, while excitatory responses to ACh were not affected. On the other hand, depressant responses to adrenoceptor agonists seemed to be resistant to α-adrenoceptor blocking agents (see Figure 4). This observation is in agreement with previous findings (Johnson *et al.*, 1969), and probably reflects the failure of α-adrenoceptor blocking agents to interact directly with β-receptors (Nickerson, 1967). Consequently, we were able to demonstrate that, on the same cell, the antagonism of excitatory responses to phenylephrine occurred when depressant responses to IPNA were not affected (see Figure 4). Occasionally a depressant response could be revealed after the abolition of the excitatory response to IPNA, suggesting an action of IPNA at both types of receptor and also the lower affinity of the antagonist for inhibitory β-receptors. The selective blockade of excitatory α-receptors may explain the observation that a depressant response to IPNA could be revealed by phenoxybenzamine on a cell which previously had not responded to IPNA; the failure of IPNA to evoke a response in the absence of phenoxybenzamine could have been due to a complete 'antagonistic agonism' (Szabadi, 1975) resulting from the activation of the functionally opposite α- and β-receptors.

The β-adrenoceptor blocking agents effectively abolished depressant responses without affecting excitatory responses to adrenoceptor agonists (see Figure 6). These antagonists occasionally unmasked excitatory responses after the abolition of depressant responses to IPNA, or when IPNA alone was without any effect, indicating the greater sensitivity of the inhibitory β-receptors. The β-receptor blocking agents, however, were also capable of antagonizing excitatory responses. This finding is in agreement with previous reports (Johnson *et al.*, 1969; Bevan *et al.*, 1974a; Bevan *et al.*, 1976a). This observation might seem to argue against our hypothesis that the excitatory responses are mediated by α-receptors. There is, however, good experimental evidence in the periphery that the conventional β-receptor blocking agents can also block α-receptors at somewhat higher concentrations than are required for selective β-receptor blockade (Patil, Tye, May, Hetey & Miyagi, 1968; Gulati, Gokhale, Parikh, Udwadia & Krishnamurty, 1969). On the basis of these observations, it might have been expected that higher ejecting currents would be needed to apply the β-adrenoceptor blocking agents in order to antagonize excitatory responses than to antagonize depressant responses. However, this was not apparent in the present results, and it is unlikely that such a difference could be detected using between-cell comparisons unless a considerably larger number of cells is studied. The differential sensitivities of inhibitory and excitatory receptors to β-adrenoceptor blocking agents may explain our previous observation that lower doses of sotalol often potentiate excitatory neuronal responses to NA, whereas higher doses have an antagonistic effect (Bevan *et al.*, 1974a; Bevan, Bradshaw & Szabadi, 1974b). The potentiation may reflect the selective blockade of masked inhibitory β-receptors whereas the antagonism may reflect the blockade of both the β-receptors and the dominant excitatory α-receptors.

Similar to the situation in many peripheral tissues (see Furchgott, 1972), α- and β-receptors mediate opposite effects on cortical neurones, the α-receptors being excitatory and the β-receptors being inhibitory. Moreover, most neurones studied provided evidence for the presence of both α- and β-receptors (e.g. opposite effects of different agonists on the same cell, see Figure 1; opposite effects to different doses of IPNA on the same cell, see Figure 2; reversal of the response by an antagonist, see Figure 3b). Although the experimental evidence presented here does not indicate *where* these receptors are localized, the simplest explanation seems to be that the two functionally opposing populations of α- and β-receptors occur on the membrane of the same cell (Szabadi & Bradshaw, 1974).

This work was supported by the North Western Regional Health Authority and the Mental Health Trust and Research Fund. We are grateful to Mrs E. Markham for technical assistance.

References

AHLQUIST, R.P. (1948). A study of the adrenotropic receptors. *Amer. J. Physiol.*, **153**, 586–600.

BESSE, J.C. & FURCHGOTT, R.F. (1976). Dissociation constants and relative efficacies of agonists acting on *alpha* adrenergic receptors in rabbit aorta. *J. Pharmac. exp. Ther.*, **197**, 66–78.

BEVAN, P., BRADSHAW, C.M., ROBERTS, M.H.T. & SZABADI, E. (1974a). The effect of microelectrophoretically applied mescaline on cortical neurones. *Neuropharmacology*, **13**, 1033–1045.

BEVAN, P., BRADSHAW, C.M. & SZABADI, E. (1974b). Potentiation and antagonism of neuronal responses to monoamines by methysergide and sotalol. *Br. J. Pharmac.*, **50**, 445P.

BEVAN, P., BRADSHAW, C.M. & SZABADI, E. (1976a). The action of microelectrophoretically applied L-3,4-dihydroxyphenylalanine (DOPA) on single cortical neurones. *Br. J. Pharmac.*, **58**, 239–246.

BEVAN, P., BRADSHAW, C.M. & SZABADI, E. (1976b). Neuronal responses to adrenoceptor agonists in the cerebral cortex: evidence for excitatory α- and inhibitory β-receptors. *Br. J. Pharmac.*, **58**, 418P.

BRADSHAW, C.M., ROBERTS, M.H.T. & SZABADI, E. (1973a). Kinetics of the release of noradrenaline from micropipettes: interaction between ejecting and retaining currents. *Br. J. Pharmac.*, **49**, 667–677.

BRADSHAW, C.M., SZABADI, E. & ROBERTS, M.H.T. (1973b). The reflection of ejecting and retaining currents in the time-course of neuronal responses to microelectrophoretically applied drugs. *J. Pharm. Pharmac.*, **25**, 513–520.

BRITTAIN, R.P., JACK, D. & RITCHIE, A.C. (1970). Recent β-adrenoceptor stimulants. In *Advances in Drug Research*, Vol. 5, ed. Harper, N.J. & Simmonds, Alma B., pp. 197–253. London and New York: Academic Press.

FURCHGOTT, R.F. (1970). Pharmacological characteristics of adrenergic receptors. *Fedn Proc.*, **29**, 1352–1361.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In *Handbook of Experimental Pharmacology*, Vol. 33, *Catecholamines*, ed. Blaschko, H. & Muscholl, E., pp. 283–335. Berlin and Heidelberg: Springer-Verlag.

GULATI, O.D., GOKHALE, S.D., PARIKH, H.M., UDWADIA, B.P. & KRISHNAMURTY, V.S.R. (1969). Evidence for sympathetic *alpha* receptor blocking action of *beta* receptor blocking agents. *J. Pharmac. exp. Ther.*, **166**, 35–43.

INNES, I.R. & NICKERSON, M. (1975). Norepinephrine, epinephrine and sympathomimetic amines. In *The Pharmacological Basis of Therapeutics*, Fifth Edition, ed. Goodman, L.S. & Gillman, A., pp. 477–513. New York: Macmillan.

JOHNSON, E.S., ROBERTS, M.H.T., SOBIESZEK, A. & STRAUGHAN, D.W. (1969). Noradrenaline sensitive cells in cat cerebral cortex. *Int. J. Neuropharmac.*, **8**, 549–566.

KRNJEVIĆ, K. & PHILLIS, J.W. (1963). Actions of certain amines on cerebral cortical neurones. *Br. J. Pharmac. Chemother.*, **20**, 471–490.

NICKERSON, M. (1967). New developments in adrenergic blocking drugs. *Ann. N.Y. Acad. Sci.*, **139**, 571–579.

PATIL, P.N., TYE, A., MAY, C., HETEY, S. & MIYAGI, S. (1968). Steric aspects of adrenergic drugs. XI. Interactions of dibenamine and *beta* adrenergic blockers. *J. Pharmac. exp. Ther.*, **163**, 309–319.

SCHILD, H.O. (1973). Receptor classification with special reference to β-adrenergic receptors. In *Drug Receptors*, ed. Rang, H.P., pp. 29–36. London and Tokyo: University Park Press.

SCHÜMANN, H.-J. & ENDOH, M. (1976). α-Adrenoceptors in ventricular myocardium: clonidine, naphazoline and methoxamine as partial α-agonists exerting a competitive dualism in action to phylephrine. *Eur. J. Pharmac.*, **36**, 413–421.

SPEDDING, M. & WEETMAN, D.F. (1972). The presence of β-adrenoceptors in the guinea-pig seminal vesicle. *Br. J. Pharmac.*, **45**, 21–28.

SZABADI, E. (1975). A theoretical model of two functionally opposite receptor populations. *Br. J. Pharmac.*, **55**, 311P.

SZABADI, E. & BRADSHAW, C.M. (1974). The role of physical and biological factors in determining the time-course of neuronal responses. *Neuropharmacology*, **13**, 537–545.

TRENDELENBURG, U. (1974). An analysis of the *alpha* and *beta* effects of isoprenaline on the isolated nictitating membrane. *Naunyn-Schmiedebergs Arc. Pharmac.*, **285**, 375–393.

(Received October 8, 1976.)