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Neuronal responses to noradrenaline in the cerebral cortex: evidence against the involvement of α_2-adrenoceptors

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1 The technique of microelectrophoresis was used to test the hypothesis that α_2-adrenoceptors are involved in mediating the excitatory responses of single neurones to noradrenaline in the somatosensory cerebral cortex of the rat.

2 In the first series of experiments the effects of two α_2-adrenoceptor antagonists, yohimbine and idazoxan (RX-781094), were compared on excitatory responses to noradrenaline, phenylephrine and acetylcholine. The response to noradrenaline was not more susceptible to antagonism by these drugs than the response to the α_1-adrenoceptor stimulant, phenylephrine. Yohimbine antagonized responses to all three agonists equally, while idazoxan antagonized responses to noradrenaline and phenylephrine equally with relative preservation of responses to acetylcholine.

3 In the second series of experiments the effects of the selective α_2-adrenoceptor stimulant, UK-14304, were examined. UK-14304 produced weak and inconsistent excitations on a small number of cells; however, most of the cells did not respond to this drug. When applied continuously using low ejection currents, UK-14304 selectively and reversibly antagonized responses to noradrenaline and phenylephrine without affecting responses to acetylcholine.

4 These results suggest that, in the somatosensory cortex of the rat, neuronal excitation to noradrenaline is unlikely to be mediated either wholly or partly by α_2-adrenoceptors. The antagonism of neuronal responses to noradrenaline and phenylephrine by idazoxan probably reflects the α_1-adrenoceptor antagonistic properties of the drug which is known to occur at higher concentrations. The low agonistic potency of UK-14304 and the antagonism of responses to noradrenaline and phenylephrine by UK-14304 suggest that this drug, like clonidine, may act as a partial agonist at α_1-adrenoceptors.

Introduction

Single cortical neurones can respond with both excitation and depression to noradrenaline applied by microelectrophoresis (for review, see Szabadi, 1979). We have previously obtained evidence that the excitatory response is mediated by α-adrenoceptors whereas the depressant response is mediated by β-adrenoceptors (Bevan *et al.*, 1977). So far as the type of α-adrenoceptor mediating the excitatory response is concerned, there is good evidence that α-adrenoceptors are involved. Thus, the selective α_1-adrenoceptor agonists methoxamine and phenylephrine evoke only excitatory responses (Bevan *et al.*, 1977; Bradshaw *et al.*, 1981a), and the response to phenylephrine can be selectively antagonized by the α_1-adrenoceptor antagonists prazosin (Bradshaw *et al.*, 1982) and haloperidol (Bradshaw *et al.*, 1983a,c).

Previous work in this laboratory suggests that the excitatory response to noradrenaline may reflect the activation not only of α_1-adrenoceptors but also of another type of excitatory receptor. Thus the neuroleptic haloperidol can discriminate between excitatory responses to noradrenaline and phenylephrine, the response to noradrenaline being less susceptible to antagonism by haloperidol than the response to phenylephrine (Bradshaw *et al.*, 1983a). Since noradrenaline, unlike phenylephrine, has affinity not only for α_1-, but also for α_2-adrenoceptors (Lavin *et al.*, 1981), the possibility arose that the 'haloperidol-resistant' component of the excitatory response to noradrenaline reflects the activation of α_2-adrenoceptors. Therefore, in the present paper we have addressed ourselves to the question whether the stimulation of α_2-adrenoceptors may also contribute

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to the excitatory neuronal response to noradrenaline.

In a previous attempt to identify the role of α_2-adrenoceptors in mediating the response to noradrenaline, we examined the effects of the relatively selective α_2-adrenoceptor stimulant, clonidine (Bradshaw *et al.*, 1982). This experiment failed to provide any evidence for the stimulation of α_2-adrenoceptors by clonidine: the weak excitatory response to clonidine could be antagonized by prazosin, and clonidine itself could antagonize excitatory responses to phenylephrine, suggesting a partial agonistic action of clonidine at α_1-adrenoceptors.

Clonidine, however, is not the ideal tool for the identification of α_2-adrenoceptors: it has been shown in smooth muscle test systems that clonidine is only a partial agonist at α_2-adrenoceptors and that it also has affinity for α_1-adrenoceptors (Schümann & Endoh, 1976; Ruffolo *et al.*, 1980). Therefore, we have re-examined the role of α_2-adrenoceptors on cortical neurones taking advantage of some selective and potent α_2-adrenoceptor antagonists and agonists which have become available since our experiment with clonidine.

In the present investigation we have used two selective α_2-adrenoceptor antagonists, yohimbine (Langer, 1980) and idazoxan (2-[2-[1, 4 benzodioxanyl]] 2-imidazoline; RX-781094) (Doxey *et al.*, 1983), to examine whether these antagonists can discriminate between neuronal excitatory responses to noradrenaline and phenylephrine. We also examined the effect of UK-14304 (5-bromo-6-[2 - imidazolin - 2 - ylamino] - quinoxaline), an imidazoline derivative that is a more selective and more potent agonist at α_2-adrenoceptors than clonidine (Cambridge, 1981).

A preliminary account of the present work has been communicated to the British Pharmacological Society (Bradshaw *et al.*, 1983b).

Methods

Male Wistar rats (230 to 270 g) were anaesthetized with halothane (0.8 to 1.0%) in oxygen. Our methods for the surgical preparation of the animals, for the manufacture of six-barrelled micropipettes (of tip diameter 3.0 to 5.0 μm), for the extracellular recording of action potentials and for the microelectrophoretic application of drugs have been described elsewhere (Bradshaw *et al.*, 1973a,b). Spontaneously active neurones were studied in the cerebral cortex (stereotaxic co-ordinates, according to König & Klippel (1963): A 4.8–6.5, L 0.9–2.4). All the drugs were applied by microelectrophoresis.

Two barrels of each micropipette contained 4.0 M NaCl, one for recording action potentials, the other for current balancing. The remaining barrels con-

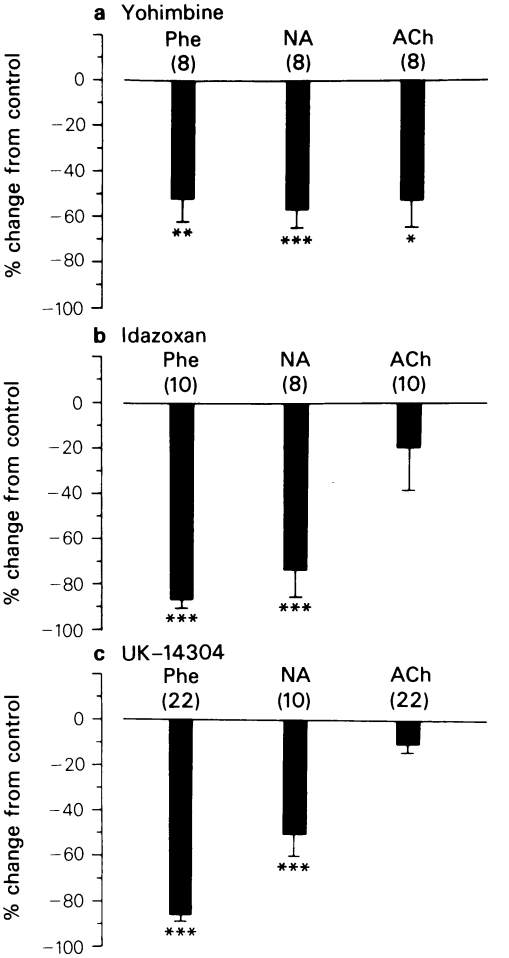

Figure 1 Summary of effects of yohimbine, idazoxan and UK-14304 on excitatory responses to phenylephrine (Phe), noradrenaline (NA) and acetylcholine (ACh). For each agonist, the length of the column represents the mean percentage change from control of the size of the response (total spike number) in the presence of the antagonist; vertical bars indicate s.e.mean. Significant changes from control values: $^*P<0.01$; $^{**}P<0.02$; $^{***}P<0.001$ (Student's t test, paired comparison). Numbers in parentheses correspond to the numbers of cells studied. (a) Effect of yohimbine on responses to phenylephrine, noradrenaline and acetylcholine. Yohimbine significantly antagonized responses to the amines and to the control agonist acetylcholine. (b) Effect of idazoxan on responses to phenylephrine, noradrenaline and acetylcholine. Idazoxan significantly antagonized responses to phenylephrine and noradrenaline but had no significant effect on responses to acetylcholine. (c) Effect of UK-14304 on responses to phenylephrine, noradrenaline and acetylcholine. UK-14304 significantly antagonized responses to phenlephrine and noradrenaline but had no significant effect on responses to acetylcholine. UK-14304 had a significantly greater effect on responses to phenylephrine than on responses to noradrenaline ($P<0.01$; t test, unpaired comparison).

tained drug solutions. The following drug solutions were used: (−)-noradrenaline bitartrate (0.05 M, pH 3.0 to 3.5); (−)-phenylephrine hydrochloride (0.05 M, pH 5.0 to 6.0); acetylcholine chloride (0.05 M, pH 4.5 to 5.5); UK-14304 tartrate (0.05 M, pH 3.4 to 3.6); yohimbine hydrochloride (0.01 M, pH 5.5 to 6.0); idazoxan hydrochloride (0.01 M, pH 6.0 to 6.5). UK-14304 tartrate was obtained from Pfizer Limited, and idazoxan hydrochloride from Reckitt & Colman Pharmaceuticals Division. Drug ions were released by positive ejecting currents. Between successive applications of agonists retaining currents of −10 nA were passed; retaining currents of −25 nA were used for the antagonists.

The agonistic effects of UK-14304 were assessed by inclusion of this compound in an ejection cycle with phenylephrine and acetylcholine. A cell was considered to be unresponsive to UK-14304 if no change in spontaneous firing rate was observed following application of UK-14304 with ejecting currents of up to 150 nA for 1 min. The relative potencies of UK-14304 and phenylephrine were estimated from current-response curves (Bradshaw *et al.*, 1982).

Antagonism studies were carried out using methods described previously (Bradshaw *et al.*, 1982). Acetylcholine was used throughout as the control agonist. The degree of antagonism of responses to the agonists was expressed as the percentage change in the size of the response from the size of the control response (Bradshaw *et al.*, 1973b). Values are expressed as mean ± s.e.mean. Statistical comparisons were made by use of Student's *t* test.

Results

Effects of α_2-adrenoceptor antagonists on responses to noradrenaline and phenylephrine

Effects of yohimbine On none of the eight cells studied did yohimbine selectively antagonize the excitatory response to noradrenaline; on all these cells responses both to the amines and to acetylcholine were almost completely abolished (Figure 1a).

Effects of idazoxan On three of the ten cells yielding consistent excitatory responses to noradrenaline, phenylephrine and acetylcholine, idazoxan antagonized the responses to the amines without affecting that to acetylcholine. An example of this observation is illustrated in Figure 2. On the remaining seven cells, the antagonism of the responses to the amines was accompanied by a reduction in the size of the response to acetylcholine. The results from all the cells are summarized in Figure 1b.

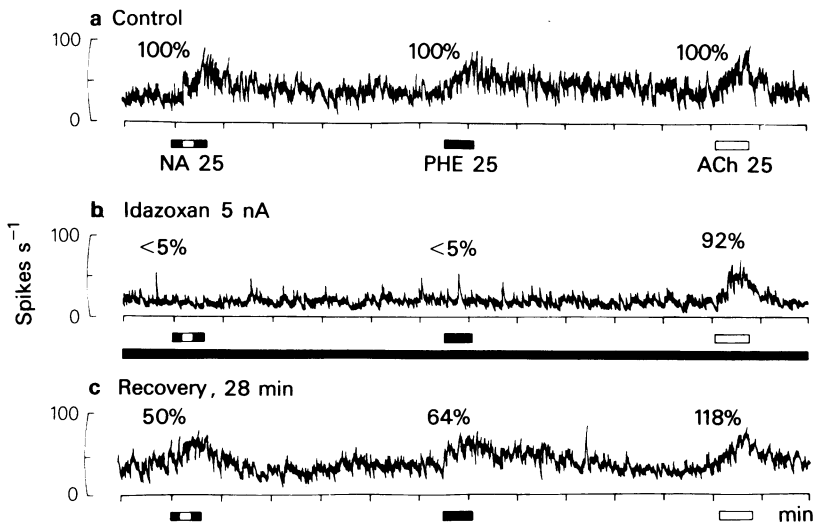

Figure 2 Effect of idazoxan on excitatory responses to noradrenaline (NA), phenylephrine (PHE) and acetylcholine (ACh). Excerpts from the ratemeter recording of the firing rate of a single cortical neurone; ordinate scale: firing rate (spikes s^{-1}); abscissa scale: running time (min). Horizontal bars below the traces indicate microelectrophoretic drug applications; numbers refer to intensities of ejecting current (nA). Numbers above the traces indicate the sizes of the responses (total spike number, %), taking the size of the control response to each agonist as 100%. (a) Control responses to the agonists; (b) responses to the agonists during the continuous application of idazoxan (5 nA). At the start of trace (b) idazoxan had been applied continuously for 47 min. The responses to noradrenaline and phenylephrine were antagonized while the response to acetylcholine was not affected. (c) Partial recovery of the responses to the amines 28 min after the application of idazoxan had been terminated.

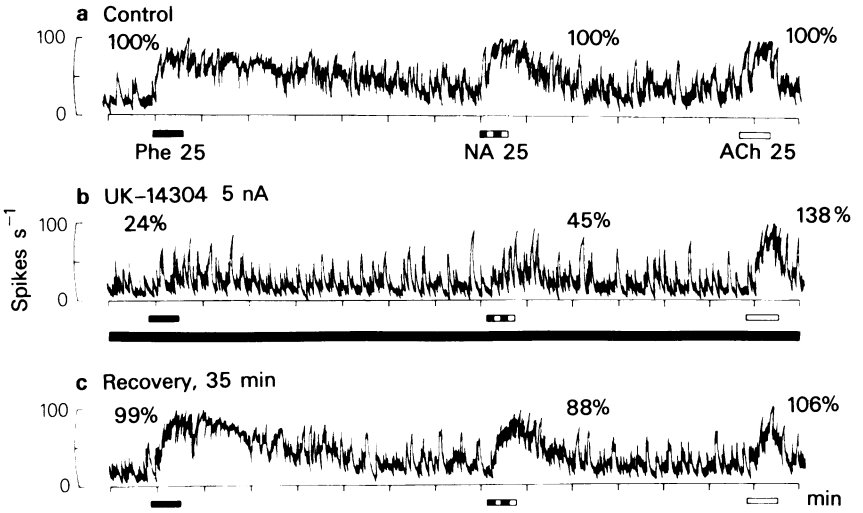

Figure 3 Effect of UK-14304 on excitatory responses to phenylephrine (Phe), noradrenaline (NA) and acetylcholine (ACh). Excerpts from the ratemeter recording of the firing rate of a single cortical neurone (convention as in Figure 2). (a) Control responses to the agonists. (b) Responses to the agonists during the continuous application of UK-14304 (5 nA). At the start of trace (b) UK-14304 had been applied continuously for 35 min. The responses to phenylephrine and noradrenaline were antagonized while the response to acetylcholine was not reduced. (c) Recovery of the responses to the amines 35 min after the application of UK-14304 had been terminated.

Effects of UK-14304

Agonistic effects UK-14304 produced weak excitation on four of the 24 phenylephrine-sensitive cells studied; the remaining 20 cells did not respond to the drug applied with ejecting currents of up to 150 nA. This weak and unreliable excitatory action of UK-14304 precluded any quantitative comparison of the relative potencies of UK-14304 and phenylephrine on all but two cells. On both of these cells, the apparent potency of phenylephrine was greater than that of UK-14304.

Antagonistic effects The ability of UK-14304 to antagonize responses to phenylephrine was investigated on 22 cells. On each of these cells the response to phenylephrine was antagonized, while responses to acetylcholine were not affected. On ten of these cells the excitatory responses to noradrenaline were also examined. The responses to noradrenaline were also antagonized, although to a somewhat lesser extent than were responses to phenylephrine.

The effects of UK-14304 on responses to phenylephrine, noradrenaline and acetylcholine on one cell are shown in Figure 3, and the effects of UK-14304 on all the cells studied are summarized in Figure 1c.

Discussion

The α_2-adrenoceptor antagonists yohimbine and idazoxan failed to discriminate between neuronal responses to noradrenaline and phenylephrine. In fact, yohimbine showed no selectivity for the responses to the amines: the responses were either unaffected or, when they were reduced, the response to acetylcholine was also diminished. Idazoxan showed a similar lack of specificity on most of the cells tested. Since yohimbine (Langer, 1980) and idazoxan (Doxey *et al.*, 1983) are relatively selective and potent α_2-adrenoceptor antagonists, this finding would argue against an α_2-adrenoceptor-mediated component in the response to noradrenaline. The antagonism of the responses to noradrenaline and phenylephrine by idazoxan, with the relative preservation of the responses to acetylcholine, on three cells, may reflect the action of idazoxan at α_1- rather than α_2-adrenoceptors, since phenylephrine is a selective α_1-adrenoceptor stimulant (Langer, 1980) and idazoxan can block α_1-adrenoceptors at concentrations higher than those required to block α_2-adrenoceptors (Doxey *et al.*, 1983b; Dabiré *et al.*, 1983).

The specific and potent α_2-adrenoceptor stimulant UK-14304 (Cambridge, 1981) showed little agonis-

tic activity in our experiment. In fact, only on a small proportion of the noradrenaline- and phenylephrine-sensitive neurones could a weak and variable excitatory effect of this drug be detected. It is unlikely that the low apparent agonistic potency of UK-14304 was due to the lack of adequate release of this drug from the micropipettes, since this drug was a potent antagonist of responses to noradrenaline and phenylephrine when applied with relatively low (5–10 nA) ejecting currents (see below). Unfortunately, the lack of availability of radioactively labelled UK-14304 precluded the direct measurement of the transport number of this drug, and the lack of availability of the hydrochloride salt prevented us from using our indirect method (Bradshaw *et al.*, 1981b) to estimate the transport number.

UK-14304 was a potent antagonist of excitatory neuronal responses to phenylephrine and noradrenaline, with no effect on responses to acetylcholine. Since phenylephrine is a specific α_1-adrenoceptor stimulant, this observation would suggest an interaction of UK-14304 with α_1-adrenoceptors. The most parsimonious explanation to account for both the weak excitatory effect and the α_1-adrenoceptor antagonistic effect of UK-14304 is that this drug, similarly to clonidine (Bradshaw *et al.*, 1982), acts as a partial agonist at α_1-adrenoceptors. Unfortunately, it was not possible to support this conclusion by an investigation of the effects of a specific α_1-adrenoceptor antagonist on the responses to UK-14304: the weak and variable nature of these responses precluded any such study. It is of interest that both clonidine (Ruffolo *et al.*, 1980) and also UK-14304 (R.R. Ruffolo, personal communication) can act as partial agonists at α_1-adrenoceptors in vascular smooth muscle.

Both clonidine (Bradshaw *et al.*, 1982) and UK-14304 (see Figure 1) showed somewhat greater antagonistic effects against responses to phenylephrine than against responses to noradrenaline. This observation is consistent with an interaction of clonidine and UK-14304 with α_1-adrenoceptors, and is in agreement with our previous finding that the response to noradrenaline has a component which is relatively resistant to antagonism by another α_1-adrenoceptor antagonist, haloperidol (Bradshaw *et al.*, 1983a). The type of receptor responsible for this haloperidol-resistant component of the response to noradrenaline remains to be identified. The possible involvement of excitatory dopamine and 5-hydroxytryptamine receptors has already been excluded (Bradshaw *et al.*, 1983a,c), and the present results strongly argue against the involvement of α_2-adrenoceptors.

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