Rapid, agonist-induced desensitization of α_2 -autoreceptors modulating transmitter release

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1 The release of previously incorporated [³H]-noradrenaline was investigated in cultures of dissociated chick or rat sympathetic neurones and in cerebrocortical slices from neonatal or adult rats. Noradrenaline, in the presence of 10 $\mu\text{mol l}^{-1}$ of the uptake inhibitor, cocaine, or the selective α_2 -adrenoceptor agonist, 5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-quinoxalinamine (UK 14,304), was applied for different periods of time in order to detect a possible time-dependence of the α_2 -adrenoceptor-mediated inhibition of electrically evoked tritium outflow.

2 In chick sympathetic neurones, stimulation-evoked overflow was reduced to 30%, 42%, or 56% of control when noradrenaline (1 $\mu\text{mol l}^{-1}$) was present for 2, 8, or 16 min, respectively. Likewise, UK 14,304 (1 $\mu\text{mol l}^{-1}$) present for these periods of time reduced ³H overflow to 35%, 51%, and 53% of control, respectively. Addition of 1 nmol l⁻¹ to 10 $\mu\text{mol l}^{-1}$ UK 14,304 for either 2 or 16 min did not produce significantly different IC₅₀ values, but the inhibitory effects were smaller with 16 min as compared to 2 min exposure at concentrations ≥ 10 nmol l⁻¹.

3 In rat sympathetic neurones, noradrenaline (100 nmol l⁻¹) reduced stimulation-evoked overflow to 33%, 56%, or 57% of control, when present for 2, 8, or 16 min, respectively. Addition of UK 14,304 (1 $\mu\text{mol l}^{-1}$) for these periods of time caused inhibition to 11%, 41%, and 46% of control. Applying UK 14,304 for either 2 or 16 min did not result in significantly different IC₅₀ values, but the inhibition induced by 16 min as compared to 2 min exposure was smaller at concentrations ≥ 10 nmol l⁻¹.

4 In cerebrocortical slices from either neonatal or adult rats, exposure to 0.1 to 1.0 $\mu\text{mol l}^{-1}$ UK 14,304 for 16 min never caused a smaller inhibition than a corresponding 3 min exposure, although various experimental conditions were investigated.

5 The results demonstrate that α_2 -adrenoceptors which regulate noradrenaline release from sympathetic neurones undergo agonist-induced desensitization within minutes. Such rapid desensitization of α_2 -autoreceptors was not detected in brain slice preparations.

Keywords: Desensitization; α_2 -adrenoceptor; noradrenaline release; sympathetic neurones; brain slices

Introduction

α_2 -Adrenoceptors control transmitter release from central and peripheral noradrenergic neurones (for review, see Starke, 1987). Effects of transmitters in many instances show desensitization which is most commonly caused by protein phosphorylation (see Haganir & Greengard, 1990). However, in the case of the α_2 -adrenoceptors, receptor desensitization has remained a controversial issue (see e.g. the discussion in Liggett *et al.*, 1992). After 10 min of agonist exposure, phosphorylation by β -adrenoceptor kinase and sequestration of α_2 -adrenoceptors have been demonstrated, paralleled in this time course by desensitization of the α_2 -adrenoceptor-induced inhibition of adenylyl cyclase activity (Liggett *et al.*, 1992). However, it has not yet been shown whether such short term desensitization occurs during α_2 -autoreceptor-mediated inhibition of transmitter release. Hence, the present study aimed at demonstrating a possible rapid, agonist-induced desensitization of α_2 -adrenoceptors which modulate noradrenaline release.

Electrically evoked tritium overflow from primary cultures of rat (Schwartz & Malik, 1993) or chick sympathetic neurones (Boehm *et al.*, 1991) labelled with [³H]-noradrenaline is markedly reduced by the activation of α_2 -adrenoceptors. In addition, these monolayer cultures virtually lack autoinhibitory feedback (e.g. Boehm *et al.*, 1991) and thus are useful preparations to study various properties of α_2 -autoreceptors. However, tissue slice preparations contain-

ing noradrenergic axon terminals of central or peripheral origin are utilized more frequently to investigate the α_2 -adrenoceptor-mediated inhibition of transmitter release (see review by Starke, 1987). The present paper examines the inhibition of electrically evoked [³H]-noradrenaline release by α_2 -adrenoceptor agonists in both types of preparations, i.e. cultures of sympathetic neurones and cerebrocortical slices. The experiments were designed to detect a possible attenuation of this inhibition induced by the time of agonist exposure.

Methods

Preparation of cell cultures and brain slices

Neurones derived either from lumbosacral paravertebral sympathetic ganglia of 12 day old chick embryos (Boehm *et al.*, 1991) or from superior cervical ganglia of 2 to 6 day old Sprague Dawley rat pups (Boehm, 1994) were dissociated and cultured as described previously. Cultures of rat neurones were used for transmitter release experiments after 3 to 4 days *in vitro*, those of chick neurones after 5 days.

The parieto-occipital cortex was removed from the brains of either the rat pups which were used for the dissection of superior cervical ganglia or adult rats (weight ≥ 250 g), and 0.35 mm thick slices were prepared with a McIlwain tissue chopper.

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Uptake and superfusion

After incubation in [3 H]-noradrenaline ($0.03 \mu\text{mol l}^{-1}$ for neuronal cell cultures, $0.07 \mu\text{mol l}^{-1}$ for brain slices) the cultures or slices were superfused with a modified Krebs Ringer bicarbonate buffer, as described previously (see Boehm *et al.*, 1994). Cultures were superfused at 25°C , slices at 37°C or 25°C . Collection of 4 min superfusate fractions was started after a 60 min washout period, and electrical field stimulations were performed after 68 min (S_1) and 96 min (S_2) of superfusion (cultures: 36 monophasic rectangular pulses, 3 Hz, 0.5 ms, 60 mA, 40 V cm^{-1} ; slices: 54 monophasic rectangular pulses, 3 Hz, 2.0 ms, 18 mA, 14 V cm^{-1}). Noradrenaline or 5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-quinoxalinamine (UK 14,304) was added to the superfusion medium at different times before S_2 (cultures: 2, 8, or 16 min; slices: 3 or 16 min) and maintained at constant concentrations until the end of experiments. Cocaine ($10 \mu\text{mol l}^{-1}$) was included in the medium for the entire superfusion period when noradrenaline was used as an agonist in order to prevent uptake of the unlabelled amine. In some experiments with brain slices yohimbine (10 nmol l^{-1}) was added to the buffer for uptake and superfusion. At the end of experiments, the radioactivity remaining in cultures or tissue slices was extracted by immersion of discs or slices in 1.2 ml 2% (v/v) perchloric acid followed by sonication. Radioactivity in extracts and collected fractions was determined by liquid scintillation counting (Beckmann LS 6500).

Calculations

The fractional rate of ^3H outflow was obtained by dividing the radioactivity of a 4 min sample by the total radioactivity of cultures at the beginning of the corresponding 4 min collection period. The rate of outflow per minute was obtained by a subsequent division by 4. Stimulation-evoked overflow was calculated as the difference between the total tritium outflow during and after the stimulation and the estimated basal outflow, which was assumed to decline linearly from the sample preceding stimulation, to the sample 8–12 min after the beginning of the stimulus. This difference was expressed as a percentage of the total radioactivity in the cultures at the beginning of the respective stimulation ($S_1\%$, $S_2\%$). Effects of α_2 -adrenoceptor agonists were evaluated by calculating ratios of the overflow evoked by S_2 and S_1 (S_2/S_1). Ratios between the arithmetic mean of S_2/S_1 values obtained with identical concentrations of UK 14,304 present for either 16 or 2 min ($[16']/[2']$) were taken as a measure of desensitization. Effects on basal ^3H outflow were estimated by the ratio of the fractional rate of outflow during the 4 min period preceding S_2 and during the 4 min period preceding S_1 (L_2/L_1).

All data are given as arithmetic means $\pm$ s.e.mean; n = number of cell culture discs or brain slices. Significance of differences between various groups was determined by the Mann-Whitney test. Concentration-response curves were fitted to the experimentally obtained data points by using the ALLFIT programme (DeLean *et al.*, 1978) which estimates the IC_{50} , the slope, and the maximal effect as well as corresponding standard errors. Moreover, the programme indicates significant differences between concentration-response curves by simultaneous fitting with shared parameters and subsequent calculation of the F -statistic on the resulting 'extra sum of squares' (DeLean *et al.*, 1978).

Drugs

Drugs were obtained from the following sources: (-)-[ring-2,5,6- ^3H]-noradrenaline ($71.7 \text{ Ci mmol}^{-1}$; NEN, Dreieich, Germany); 5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-quinoxalinamine (UK 14,304; Research Biochemicals, Natick, MA, U.S.A.); (-)-noradrenaline HCl, cocaine HCl (Sigma,

München, Germany); yohimbine HCl (Merck, Darmstadt, Germany).

UK 14,304 was initially dissolved at 1 mmol l^{-1} in 0.1 mol l^{-1} HCl; all other drugs were dissolved in distilled water. All drugs were diluted to the final concentrations in superfusion medium.

Results

Cultures of chick sympathetic neurones

Values of spontaneous and stimulation-evoked tritium outflow from cultures of chick sympathetic neurones are shown in Table 1. Inclusion of $10 \mu\text{mol l}^{-1}$ cocaine in the medium significantly enhanced electrically induced overflow, but failed to alter fractional basal outflow (Table 1). These results corroborate the previously described enhancement by cocaine of stimulation-evoked noradrenaline overflow from chick sympathetic neurones (Boehm *et al.*, 1991). Noradrenaline (in the presence of $10 \mu\text{mol l}^{-1}$ cocaine) or the selective α_2 -adrenoceptor agonist, UK 14,304 (Cambridge, 1981), both at $1 \mu\text{mol l}^{-1}$, significantly reduced stimulation-evoked overflow from chick sympathetic neurones ($P \leq 0.001$ vs. corresponding controls). The inhibition caused by each of the two was significantly smaller with 16 min than with 2 min exposure. However, effects did not significantly differ if agonists were present for 8 min as compared to 16 min before S_2 (Figure 1a).

Addition of 1 nmol l^{-1} to $10 \mu\text{mol l}^{-1}$ UK 14,304 either 2 or 16 min before S_2 yielded concentration-response curves for the inhibition of stimulation-evoked tritium overflow (Figure 1b) not differing ($P > 0.05$) with respect to IC_{50} values which were $16.6 \pm 9.3 \text{ nmol l}^{-1}$ for 16 min and $6.5 \pm 2.3 \text{ nmol l}^{-1}$ for 2 min agonist exposure. However, the extent of inhibition by UK 14,304 at concentrations of 10 nmol l^{-1} and above was significantly smaller with 16 min as compared to 2 min exposure. The ratio of mean S_2/S_1 values obtained with 16 and 2 min exposures to UK 14,304 ($[16']/[2']$), taken as a measure of desensitization, displayed a concentration-dependent increase which was half maximal at around 10 nmol l^{-1} (Figure 1b). Thus, UK 14,304 induced desensitization and inhibition of stimulation-evoked overflow in the same range of concentrations.

Neither noradrenaline in the presence of cocaine nor UK 14,304, at any of the above concentrations or periods of

Table 1 Fractional basal tritium outflow per minute and electrically evoked overflow ($S_1\%$) from superfused cultures of chick or rat sympathetic neurones labelled with [^3H]-noradrenaline

Species	Cocaine ($\mu\text{mol l}^{-1}$)	Basal outflow (min^{-1})	Stimulated overflow ($S_1\%$)	n
Chick	0	0.0016 ± 0.0001	1.58 ± 0.17	119
	10	0.0019 ± 0.0002	$1.89 \pm 0.14^*$	24
Rat	0	0.0017 ± 0.0001	0.41 ± 0.02	90
	10	0.0015 ± 0.0001	$0.80 \pm 0.04^{***}$	23

After labelling, neurones were superfused, 4 min fractions of superfusate were collected, and electrical field stimulation (36 pulses at 3 Hz, 0.5 ms, 60 mA) was performed after 68 (S_1) and 96 min (S_2) of superfusion. Data were obtained either under control conditions or in the presence of cocaine ($10 \mu\text{mol l}^{-1}$) which was included in the medium for the entire superfusion period. The basal ^3H efflux under control conditions corresponded to $0.027 \pm 0.001 \text{ nCi min}^{-1}$ in chick and to $0.076 \pm 0.004 \text{ nCi min}^{-1}$ in rat neurones. Stimulation-evoked overflow equalled $0.263 \pm 0.014 \text{ nCi}$ per stimulation period in chick and $0.204 \pm 0.012 \text{ nCi}$ per stimulation period in rat sympathetic neurones, respectively.

* $P \leq 0.05$; *** $P \leq 0.001$ vs. corresponding controls, respectively.

exposure, caused significant alterations of spontaneous tritium efflux from chick sympathetic neurones (not shown).

Cultures of rat sympathetic neurones

Fractional basal efflux from rat sympathetic neurones was similar to spontaneous tritium outflow from chick neurones. However, electrically induced overflow was much smaller in rat than in chick sympathetic neurones (Table 1). In accordance with the results obtained with chick neurones, stimulation-evoked overflow was markedly enhanced in the presence of cocaine ($10 \mu\text{mol l}^{-1}$) without changes in spon-

taneous outflow (Table 1). Noradrenaline ($0.1 \mu\text{mol l}^{-1}$) in the presence of cocaine ($10 \mu\text{mol l}^{-1}$) as well as UK 14,304 ($1 \mu\text{mol l}^{-1}$) reduced stimulation-evoked overflow ($P \leq 0.001$ vs. controls) to a higher degree when added 2 min before S_2 than when added 16 or 8 min before S_2 (Figure 2a).

The concentration-response curves for the reduction of stimulation-evoked overflow by either 16 or 2 min exposure to UK 14,304 (Figure 2b) displayed IC_{50} values that were not significantly different (2 min: $6.1 \pm 1.7 \text{ nmol l}^{-1}$; 16 min: $17.9 \pm 7.5 \text{ nmol l}^{-1}$; $P > 0.05$), although the effects of 16 min exposure were significantly smaller at concentrations $\geq 10 \text{ nmol l}^{-1}$ (Figure 2b). Similar to the results obtained with chick neurones, the ratio of mean S_2/S_1 values after 16 and 2 min exposures to UK 14,304 ($[16']/[2']$) showed a concentration-dependent increase that was half maximal at around 10 nmol l^{-1} (Figure 2b). Thus, in rat sympathetic neurones, the potency of UK 14,304 was about the same for the induction of desensitization as for the reduction of electrically evoked overflow.

Spontaneous ^3H outflow from rat sympathetic neurones was not affected by $0.1 \mu\text{mol l}^{-1}$ noradrenaline at any period of exposure, but slightly altered in the presence of some concentrations of UK 14,304 added 2, 8, or 16 min before S_2 (not shown).

Figure 1 Effects of noradrenaline and UK 14,304 on electrically evoked tritium overflow from cultures of chick sympathetic neurones labelled with [^3H]-noradrenaline after various periods of agonist exposure. After labelling, neurones were superfused, and tritium overflow was triggered by electrical field stimulation (36 pulses at 3 Hz) after 68 min (S_1) and 96 min (S_2) of superfusion. (a) The indicated S_2/S_1 ratios were obtained under control conditions (C) or after addition of either $1 \mu\text{mol l}^{-1}$ noradrenaline (NA) or $1 \mu\text{mol l}^{-1}$ UK 14,304 (UK) to the superfusion medium 2, 8, or 16 min before S_2 . The data with noradrenaline as agonist and corresponding controls were obtained with cocaine ($10 \mu\text{mol l}^{-1}$) in the superfusion medium. The columns show arithmetic means $\pm$ s.e. mean of 6 to 9 observations. All S_2/S_1 ratios determined in the presence of α_2 -adrenoceptor agonists were significantly different from corresponding controls at $P \leq 0.001$. Significantly different from the value obtained after a 2 min exposure to the same agonist at $*P \leq 0.05$ and $**P \leq 0.01$, respectively. (b) The indicated concentrations of UK 14,304 were added to the superfusion medium 2 (○) or 16 min (□) before S_2 , and effects of UK 14,304 were evaluated by calculating S_2/S_1 ratios. Values shown are arithmetic means $\pm$ s.e. mean of 6 to 9 observations. $**P \leq 0.01$ vs. the S_2/S_1 ratio obtained with a 16 min application of the same concentration of UK 14,304. As a measure of desensitization, the ratio between mean S_2/S_1 values obtained with 16 and 2 min exposures ($[16']/[2']$) to the indicated concentrations of UK 14,304 is shown by (▲).

Figure 2 Effects of noradrenaline and UK 14,304 on electrically evoked tritium overflow from cultures of rat sympathetic neurones labelled with [^3H]-noradrenaline after various periods of agonist exposure. (a) Noradrenaline (NA) $0.1 \mu\text{mol l}^{-1}$ or UK 14,304 (UK) $1 \mu\text{mol l}^{-1}$ were added to the superfusion medium 2, 8, or 16 min before S_2 . All S_2/S_1 ratios determined in the presence of α_2 -adrenoceptor agonists were significantly different from corresponding controls (C) at $P \leq 0.001$. (b) UK 14,304 was added to the superfusion medium 2 (○) or 16 min (□) before S_2 . Values shown are arithmetic means $\pm$ s.e. mean of 6 observations. $*P \leq 0.05$, $**P \leq 0.01$ vs. the S_2/S_1 ratio obtained with a 16 min exposure to the same concentration of UK 14,304. See legend to Figure 1 for further details.

Brain slices

Values of basal tritium outflow and of stimulation-evoked overflow from cerebrocortical slices from neonatal rats are given in Table 2. Addition of $0.3 \mu\text{mol l}^{-1}$ UK 14,304 either 3 or 16 min before S_2 caused significant reductions in electrically induced overflow from neonatal rat brain slices ($P \leq 0.001$ vs control). In contrast to the data obtained with dissociated neurones, however, there was no difference between the effects of these two periods of exposure (Table 3). Assuming that the desensitization caused by addition of an agonist to the superfusion medium might be occluded by effects of the biophase concentration of endogenous noradrenaline, experiments were repeated in presence of 10 nmol l^{-1} of the α_2 -adrenoceptor antagonist, yohimbine: as expected, yohimbine significantly enhanced stimulation-evoked overflow leaving the basal rate of ^3H outflow unaltered (Table 2). However, instead of revealing a decreased effect, yohimbine rather increased the inhibitory effect of 16 min (compared to 3 min) exposure to UK 14,304 (Table 3).

One major difference between the experiments on cell cultures and brain slices was the temperature which was 37°C for brain slices and 25°C for neuronal cell cultures. In order to exclude the possibility that this was the reason for the lack of desensitization in cerebrocortical slices, experiments on

cortex slices from neonatal rats were repeated at 25°C . This decrease in temperature reduced spontaneous and stimulation-evoked outflow (Table 2) and increased the inhibitory effect of UK 14,304 (Table 3). However, even at 25°C no desensitization was seen in cortex slices from neonatal rats. On the contrary, at this temperature $0.3 \mu\text{mol l}^{-1}$ UK 14,304 was much more effective when present for 16 instead of 3 min (Table 3).

Cerebrocortical slices derived from adult rats showed a similar outflow of tritium as slices obtained from neonatal rats. Stimulation-evoked overflow, however, was markedly smaller in brain slices from adult rats (Table 2). As in cortex slices from neonatal rats, 0.1 or $1.0 \mu\text{mol l}^{-1}$ UK 14,304 significantly reduced electrically induced tritium outflow ($P \leq 0.05$ vs. control). There was no difference between the inhibition caused by 3 and 16 min exposure to UK 14,304 (Table 3). Lowering the temperature from 37°C to 25°C , apart from reducing basal outflow (Table 2), resulted again in a smaller inhibitory effect of the agonist added 3 min before S_2 compared to the 16 min period of exposure (Table 3).

Spontaneous ^3H outflow from either neonatal or adult rat cortex slices was not affected by UK 14,304, whether added 3 or 16 min before S_2 ($P > 0.05$ vs. corresponding controls; data not shown).

Table 2 Fractional basal tritium outflow per minute and electrically evoked overflow ($S_1\%$) from superfused cerebrocortical slices obtained either from neonatal (≤ 6 d) or from adult rats and labelled with [^3H]-noradrenaline

Preparation	Condition	Basal outflow (min^{-1})	Stimulated overflow ($S_1\%$)	n
Neonatal rat	37°C	0.0037 ± 0.0001	8.16 ± 0.28	36
Cortex	$37^\circ\text{C}/\text{Yoh}$	0.0037 ± 0.00004	$9.95 \pm 0.4^{***}$	24
	25°C	$0.0016 \pm 0.0001^{***}$	$4.34 \pm 0.28^{***}$	22
Adult rat	37°C	0.0034 ± 0.0001	1.64 ± 0.19	21
Cortex	25°C	$0.0017 \pm 0.0001^{***}$	1.32 ± 0.03	12

After labelling, cortex slices were superfused at the indicated temperatures (25°C or 37°C), 4 min fractions of superfusate were collected, and electrical field stimulation (54 pulses at 3 Hz, 2.0 ms, 18 mA) was performed after 68 (S_1) and 96 min (S_2) of superfusion. Data were obtained either under control conditions or in the presence of 10 nmol l^{-1} yohimbine ($37^\circ\text{C}/\text{Yoh}$) which was included in the medium for uptake and superfusion. The basal ^3H efflux under control conditions at 37°C equalled $0.152 \pm 0.006 \text{ nCi min}^{-1}$ in neonatal rat cortex and $0.229 \pm 0.011 \text{ nCi min}^{-1}$ in adult rat cortex. Stimulation-evoked overflow corresponded to $3.483 \pm 0.232 \text{ nCi}$ per stimulation period in neonatal and to $1.152 \pm 0.157 \text{ nCi}$ per stimulation period in adult rat cortex, respectively. $^{***}P \leq 0.001$ vs. corresponding controls.

Discussion

Conclusive evidence for agonist-induced desensitization of α_2 -adrenoceptors involved in the modulation of transmitter release has been lacking. A recent study (Fuder & Schwarz, 1993) indicated a decrease in the α_2 -adrenoceptor-mediated inhibition of stimulation-evoked noradrenaline release after 30 min pre-exposure to supramaximal agonist concentrations followed by extended (≥ 50 min) washout periods. However, whether the application of α_2 -adrenoceptor agonists could cause desensitization within only a few minutes was not investigated.

Hence, the present results show for the first time that α_2 -autoreceptors involved in the feedback inhibition of transmitter release may undergo agonist-induced desensitization within minutes. This desensitization was obvious in superfused cultures of sympathetic neurones. We observed inhibition of transmitter release after 2 min exposure to maximal concentrations of UK 14,304 down to about 15% of control in rat and to approximately 30% of control in chick sympathetic neurones. Previous experiments with these cell cultures indicated considerably smaller maximal effects of UK 14,304 (50% of control in rat, Schwartz & Malik, 1993; 55% of control in chick, Boehm *et al.*, 1994), most likely because extended exposure (≥ 10 min) to the α_2 -adrenoceptor agonist had been used in these studies.

Table 3 Effects of 3 or 16 min exposure to UK 14,304 on electrically evoked tritium overflow from superfused cerebrocortical slices obtained either from neonatal (≤ 6 d) or from adult rats and labelled with [^3H]-noradrenaline

Preparation	Condition	UK 14,304 ($\mu\text{mol l}^{-1}$)	S_2/S_1		n
			3 min	16 min	
Neonatal rat	37°C	0.3	0.33 ± 0.03	0.27 ± 0.02	14
Cortex	$37^\circ\text{C}/\text{Yoh}$	0.3	0.38 ± 0.03	$0.21 \pm 0.03^{**}$	7
	25°C	0.3	0.22 ± 0.02	$0.08 \pm 0.01^{***}$	11
Adult rat	37°C	0.1	0.31 ± 0.03	0.18 ± 0.02	3
Cortex	37°C	1.0	0.17 ± 0.03	0.11 ± 0.01	4
	25°C	1.0	0.31 ± 0.02	$0.24 \pm 0.02^*$	4

The indicated concentrations of UK 14,304 were added to the superfusion medium 3 or 16 min before S_2 . The data were obtained either under control conditions (37°C), in presence of 10 nmol l^{-1} yohimbine ($37^\circ\text{C}/\text{Yoh}$), or at 25°C . S_2/S_1 values obtained under control conditions were 0.99 ± 0.01 ($n = 8$) in neonatal rat cortex and 0.78 ± 0.05 ($n = 5$) in adult rat cortex. All data obtained in the presence of UK 14,304 were significantly different from these control values at $P \leq 0.001$ for neonatal and at $P \leq 0.05$ for adult rat cerebrocortical slices. $^*P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$ vs. the corresponding 3 min exposure, respectively.

Although peripheral noradrenergic neurones of neonatal rats showed a marked agonist-induced desensitization of α_2 -autoreceptors, parieto-occipital cortex slices derived from the same rats did not display this phenomenon under conventional experimental conditions. One reason for this obvious lack of rapid, agonist-induced desensitization could have been previous desensitization caused by the accumulation of endogenously released noradrenaline. Hence, prevention of this effect of endogenous noradrenaline by an α_2 -adrenoceptor antagonist should uncover agonist-induced desensitization also in brain slices. However, in the presence of 10 nmol l^{-1} yohimbine, the inhibition of electrically evoked transmitter release after 16 min exposure to UK 14,304 was significantly larger (instead of being smaller) than the inhibition after 3 min exposure. Likewise, reduction of the temperature used for the cell culture experiments from 37°C to the 25°C did not promote desensitization, but rather increased the effect of long term (16 min) agonist exposure. These results obtained with brain tissue of neonatal rats were corroborated by results obtained in experiments with cerebrocortical slices derived from mature animals. In this tissue, the inhibitory effects of 3 or 16 min exposure to UK 14,304 did not differ significantly when assessed in experiments performed at 37°C . Lowering the temperature to 25°C resulted again in an increased effect of the long term agonist exposure, just as described for the brain tissue of neonatal rats.

It should be borne in mind, however, that both manipulations, inclusion of yohimbine (10 nmol l^{-1}) in the superfusion medium as well as lowering the temperature, which aimed at optimizing the conditions for the detection of autoreceptor desensitization, may have selectively impeded the effects of short (versus long) term agonist exposure: (a) in cerebrocortical slice preparations, blockade of α_2 -adrenoceptors by yohimbine has previously been shown to facilitate stimulation-evoked transmitter release even if yohimbine was applied for only 10 min followed by more than 2 h of washout (see Figure 5 in Hölting & Starke, 1986). Hence, removal of yohimbine from the sites of action in superfused brain slice preparations apparently proceeds very slowly. This feature of yohimbine may have retarded the occupancy of receptors by agonists in the present study. (b) In superfusion experiments on rat brain slices, reduction of the temperature from 37°C to 23°C caused marked alterations in synaptic morphology, with the higher temperature yielding a greater number of freely accessible presynaptic nerve terminals (Cichini *et al.*, 1986). Hence, α_2 -adrenoceptor agonists added to the superfusion medium presumably reach their sites of action more slowly at 25°C than at 37°C . Taken together, the

detection of rapid agonist-induced events in brain slices may be limited by the fact that a time period in the range of several minutes is required to achieve homogeneous drug concentrations throughout tissue samples. Thus, the present failure to demonstrate an agonist-induced desensitization within the time course of a few minutes in rat brain slices does not necessarily indicate that central α_2 -adrenoceptors involved in the modulation of transmitter release do not undergo desensitization. Postsynaptically located α_2 -adrenoceptors of the rat central nervous system have previously been shown to be subject to desensitization in response to long term (14 days) agonist exposure *in vivo* (Finberg & Kopin, 1987). However, in slices of the rabbit hypothalamus, the inhibition of [^3H]-noradrenaline release induced by exogenous noradrenaline added for either 8 or 20 min did not differ, whereas the dopamine-induced inhibition completely desensitized within this time course (Galzin & Langer, 1985).

α_2 -Adrenoceptors which regulate adenylyl cyclase activity desensitize with a similar time course (Liggett *et al.*, 1992) to the autoreceptors of sympathetic neurones which inhibited transmitter release in the present study. However, adenylyl cyclase is not involved in the inhibition of transmitter release in any of the preparations used in this study, at least when α_2 -adrenoceptor agonists were applied for 6 min or more (rat occipital cortex: Ong *et al.*, 1991; rat sympathetic neurones: Schwartz & Malik, 1993; chick sympathetic neurones: Boehm *et al.*, 1994). Hence, the α_2 -adrenoceptors of sympathetic neurones probably operate and desensitize independently of adenylyl cyclase. Alternatively, there may be two mechanisms of inhibition, one involving, the other not involving adenylyl cyclase. Only the mechanism involving adenylyl cyclase may then desensitize rapidly, making the neurones less sensitive to α_2 -adrenoceptor activation after a few minutes.

The detection of short term agonist-induced desensitization of the α_2 -adrenoceptor-mediated inhibition of noradrenaline release in sympathetic neurones derived from two species, points to the general importance of this phenomenon at least in postganglionic sympathetic neurotransmission. The present study failed to detect a similar mechanism in rat cerebrocortical slices. It therefore remains open whether rapid agonist-induced desensitization is a universal characteristic of α_2 -autoreceptors involved in the regulation of transmitter release or whether it can be assigned to discrete nervous structures or to certain receptor subtypes.

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