Subclassification of release-regulating α_2 -autoreceptors in human brain cortex

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1 Release-regulating α_2 -autoreceptors in human brain were characterized pharmacologically in cortical slices from patients undergoing neurosurgery to remove subcortical tumours; the slices were prelabelled with [³H]-noradrenaline ([³H]-NA) and stimulated electrically (3 Hz, 2 ms, 24 mA) under superfusion conditions.

2 The stimulus-evoked tritium overflow was almost totally Ca^{2+} -dependent and tetrodotoxin-sensitive.

3 Clonidine and oxymetazoline 0.01 to 1 μM inhibited in a concentration-dependent manner the evoked overflow of tritium. The two drugs were equipotent ($\text{EC}_{50} = 0.03 \mu\text{M}$) and their maximal effect was approx. 45%. Phenylephrine and methoxamine, up to 1 μM , did not affect tritium overflow.

4 Yohimbine (0.01–0.1 μM) shifted the concentration-response curve of clonidine to the right. The calculated pA_2 value was 8.29.

5 Prazosin and 2-[2-[4-(*o*-methoxyphenyl)piperazine-1-yl]ethyl]-4,4-dimethyl-1,3(2H,4H)-isoquinoline-dione (AR-C 239), tested at 0.3 μM , did not modify the concentration-response curve of clonidine.

6 The effect of clonidine was antagonized by (+)-mianserin ($\text{pA}_2 = 7.74$), but not by up to 0.3 μM of the (–)-enantiomer. The concentration-response curve of clonidine was shifted to the right by the novel α_2 -adrenoceptor antagonist, 5-chloro-4-(1-butyl-1,2,5,6-tetrahydropyridin-3-yl)-thiazole-2-amine (Z)-2-butenedioate (1:1) salt (ORG 20350) ($\text{pA}_2 = 7.55$).

7 Yohimbine, (+)-mianserin and ORG 20350, but not prazosin and (–)-mianserin, increased the electrically-evoked tritium overflow, suggesting that autoreceptors may be tonically activated by endogenous NA.

8 Desipramine (1 μM) increased evoked tritium overflow from human cortex slices. The effect of clonidine (0.01–1 μM) on the evoked overflow of tritium was reduced in presence of 1 μM desipramine.

9 It is proposed that autoregulation of NA release can occur in human cerebral cortex. The process involves activation of α_2 -adrenoceptors which may be either the α_{2A} or the α_{2D} subtype.

Keywords: Human cerebral cortex; noradrenaline release; noradrenaline autoreceptors; α_2 -adrenoceptor subtypes; clonidine; desipramine

Introduction

Our knowledge of noradrenaline (NA) release and of its autoregulation in the central nervous system originates essentially from experiments carried out with laboratory animals (see, for a review, Starke, 1987). The existence of NA autoreceptors in human brain was reported only in a recent study in which the subtype to which they belong was not determined (Feuerstein *et al.*, 1990).

Fresh tissues are required for release experiments and this poses obvious difficulties in 'normal' human brain specimens. On the other hand, the possibility of species differences between humans and laboratory animals appears more and more likely due to the surprising heterogeneity of receptors which has emerged in the recent years. Experiments with human brain tissue, hence, seem necessary.

Substantial pharmacological evidence supports the existence of α_2 -adrenoceptor heterogeneity in animals. Such evidence comes from functional (Doxey & Everitt, 1977; Dubocovich, 1979; Raiteri *et al.*, 1983; Alabaster *et al.*, 1986; Kapocsi *et al.*, 1987; Ruffolo *et al.*, 1987; Connaughton & Docherty, 1990; Akers *et al.*, 1991; Maura *et al.*, 1992) as well as from binding (Bylund, 1981, 1985; Cheung *et al.*,

1982; Michel *et al.*, 1989; Brown *et al.*, 1990; Uhlén & Wikberg, 1991) studies. Multiple α_2 -adrenoceptors in human tissues were predicted on the basis of molecular biology studies (Kobilka *et al.*, 1987; Regan *et al.*, 1988).

In this functional study we have characterized pharmacologically the NA autoreceptor involved in the negative feedback regulation of the release of the catecholamine in the human cerebral cortex.

Methods

Characteristics of human brain specimens

Samples of human cerebral cortex were obtained from patients undergoing neurosurgery. The tissues used had to be removed by the surgeon to reach deeply located tumours. After premedication with atropine and meperidine, anaesthesia was induced with thiopentone and maintained with 70% nitrous oxide, 30% oxygen and 0.5–1% isoflurane. Pancuronium was employed to obtain muscular relaxation. The samples removed represented parts of frontal (4), temporal (11), and parietal (3) lobes and were obtained from 7 male and 11 female patients (aged 42–72 years). The tissues were obtained and processed separately on different days.

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Preparation of slices

Immediately after removal, the tissue was placed in a physiological salt solution (see below) kept at 0–4°C and cortical slices were prepared within 60 min. Slices (0.4 mm thick) perpendicular to the surface were prepared by use of a McIlwain tissue chopper after cubic pieces had been obtained from human specimens. In some experiments (effect of clonidine in the presence of desipramine and Ca^{2+} - and tetrodotoxin-dependence) surface-parallel slabs, 0.4 mm thick, were cut from the human brain cortex samples after the superficial 0.3 mm had been removed. Slices of 5 mm diameter were punched out from the slabs. The results obtained did not depend on the method of slice preparation.

Release experiments

The slices were incubated with $0.1 \mu\text{M}$ [^3H]-NA, in the presence of $0.3 \mu\text{M}$ citalopram, for 15 min at 37°C in 5 ml of a medium having the following composition (mM): NaCl 125, KCl 3, MgSO_4 1.2, CaCl_2 1.2, Na_2HPO_4 1, NaHCO_3 22, glucose 10 (aerated with 95% O_2 and 5% CO_2), pH 7.2–7.4. After washing with tracer-free medium, one slice was transferred to each of 12 parallel superfusion chambers equipped with platinum electrodes 20 mm apart (Reichenbacher *et al.*, 1982) and superfused for 155 min at a rate of 1 ml min^{-1} . After 55 min of superfusion to achieve a constant baseline of tritium efflux, 5-min fractions were collected until the end of the experiment. The slices were then solubilized in 0.5 ml of Soluene 350 (Canberra Packard, Milan, Italy) and tritium in the superfusate samples and solubilized tissue was measured by liquid scintillation counting.

Continuous electrical stimulation with biphasic square pulses (24 mA, 3 Hz, 2 ms) started at $t = 65 \text{ min}$ ($t = 0$ being the start of superfusion), after the second fraction had been collected (see Figure 1). Some unstimulated slices were superfused in parallel in order to estimate the basal efflux of tritium and the effects of drugs on the spontaneous outflow.

The NA receptor agonists, clonidine, oxymetazoline, phenylephrine and methoxamine were added to the superfusion medium after the peak of the electrically-evoked

tritium efflux was reached (at $t = 75 \text{ min}$, see Figure 1). The agonist concentrations were then increased every 20 min (at $t = 95, 115, 135 \text{ min}$) in order to construct a cumulative concentration-response curve (Frankhuysen & Mulder, 1982; Richards, 1985; Bonanno & Raiteri, 1987). The NA receptor antagonists, yohimbine, prazosin, 2-[4-(*o*-methoxyphenyl)piperazine-1-yl]ethyl-4,4-dimethyl-1,3 (2H,4H)-isoquinoline-dione (AR-C 239), (+)-mianserin, (–)-mianserin, 5-chloro-4-(1-butyl-1,2,5,6-tetrahydropyridin-3-yl)-thiazole-2-amine (Z)-2-butenedioate (1:1) salt (ORG 20350) and the NA uptake inhibitor, desipramine, when used, were present throughout the experiment. Controls in standard medium or in the presence of desipramine or antagonists but without agonists were always run in parallel in the same experiment so that each experimental condition had its own control group.

In some experiments, slices were superfused from $t = 75$ to $t = 115 \text{ min}$ with a Ca^{2+} -free medium or in the presence of $1 \mu\text{M}$ tetrodotoxin.

Calculations

The efflux of radioactivity collected in each fraction was calculated as a percentage of the total radioactivity present in the tissue at the onset of the fraction considered. The evoked overflow in each fraction was calculated by subtraction, from the efflux in the particular fraction, of the respective basal efflux. The basal efflux was calculated from the efflux of each slice in the fraction preceding the stimulation ($t = 60$ – 65 min), assuming a decline similar to that of unstimulated slices processed in the same experiment. The evoked overflow in the fraction F_1 ($t = 70$ – 75 min) and that in every fourth fraction collected after the addition of the various agonist concentrations (F_2, F_3, F_4, F_5) was calculated (see Figure 1). Effects of the various agonists on the electrically-evoked overflow of tritium were evaluated by forming the ratios $F_2/F_1, F_3/F_1, F_4/F_1, F_5/F_1$. These ratios were compared to the corresponding ratios obtained under control conditions (i.e. in standard medium or in the presence of the antagonist or desipramine, as appropriate). In each experiment the average of appropriate F_n/F_1 values from the two superfusion chambers containing the same drug were compared to the average value of the two control chambers run in the same experiment.

The EC_{50} values for the agonists (alone or in the presence of an antagonist) were determined from a curve obtained using a function fitting routine (software Sigma Plot, version 4.0). The following four parameter logistic equation was used:

$$Y = a + ((b-a)/(1 + (10^c/10^d)^d))$$

where (b) is the maximum effect; (c) the EC_{50} ; (d) the slope of the curve. The minimum effect of an agonist (a) was always considered constant (zero % inhibition). Each EC_{50} value was the average of n EC_{50} s calculated in the different experiments.

The pA_2 values of (+)-mianserin and ORG 20350 were calculated according to Furchgott (1972, page 290); the values obtained with the two concentrations of antagonist used were averaged. The pA_2 value of yohimbine was calculated according to Arunlakshana & Schild (1959), using three concentrations of the antagonist. The antagonism was considered competitive if the slope was not significantly different from unity.

The effects of the adrenoceptor antagonists and of desipramine, shown in Figure 6, were calculated as follows: $[(F_1\text{drug} - F_1\text{control})/F_1\text{control}] \times 100$ where $F_1\text{drug}$ and $F_1\text{control}$ was the depolarization-evoked overflow of tritium in the fraction collected at $t = 70$ – 75 min in the presence of the antagonist and in standard medium, respectively.

Student's t test was used to analyze the significance of the difference between two means.

Figure 1 Tetrodotoxin- and Ca^{2+} -dependence of tritium overflow evoked by continuous electrical stimulation of human brain cortex slices. Slices prelabelled with [^3H]-noradrenaline ([^3H]-NA) were superfused. Electrical stimulation (24 mA, 3 Hz, 2 ms) was applied from $t = 65 \text{ min}$ to $t = 155 \text{ min}$. Five-min fractions were collected and the radioactivity present in each fraction was expressed as a percentage of the total tritium content of the tissue at the onset of the fraction considered. As indicated in the figure, tetrodotoxin-containing or Ca^{2+} -free medium was introduced from $t = 75 \text{ min}$ to $t = 115 \text{ min}$. Points represent the mean of three experiments in duplicate. The s.e.mean (not shown) was always within 10% of the respective mean value. (■) Experiments with electrical stimulation; (□) experiments with electrical stimulation and $1 \mu\text{M}$ tetrodotoxin; (○) experiments with electrical stimulation and Ca^{2+} -free medium; (●) experiments without electrical stimulation.

Drugs

(-)-(7,8-³H)-noradrenaline (specific activity 32 Ci mmol⁻¹) was obtained from Amersham Radiochemical Centre (Buckinghamshire); (-)-phenylephrine hydrochloride, yohimbine hydrochloride and tetrodotoxin from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). The following drugs were gifts from the companies indicated: clonidine hydrochloride (Boehringer Ingelheim, Florence, Italy); (±)-methoxamine hydrochloride (Wellcome Research Laboratories, Beckenham); citalopram hydrobromide (Lundbeck, Copenhagen, DK); desipramine hydrochloride (Ciba Geigy, Basel, Switzerland); (+)-mianserin maleate, (-)-mianserin maleate and 5-chloro-4-(1-butyl-1,2,5,6-tetrahydropyridin-3-yl)-thiazole-2-amine (Z)-2-butenedioate (1:1) salt (ORG 20350) (Organon, Oss, The Netherlands); 2-[2-[4-(o-methoxyphenyl) piperazine-1-yl] ethyl]-4,4-dimethyl-1,3(2H,4H)-isoquinolinedione (AR-C 239) (Karl Thomae, Biberach, Germany); oxymetazoline hydrochloride (Avondale Chemical, Wicklow, Ireland); prazosin hydrochloride (Pfizer, New York, U.S.A.).

Prazosin was dissolved in ethanol and (+)- and (-)-mianserin in 0.1 M HCl. Dilution to 1 mM was made with distilled water. Other compounds were first dissolved (1 mM) in distilled water. Final dilutions were made with standard medium.

Results

Ca²⁺-dependence and tetrodotoxin-sensitivity of electrically-evoked tritium overflow

Under continuous field stimulation, the evoked ³H-overflow from human cerebral cortex slices prelabelled with [³H]-NA was largely sensitive to tetrodotoxin (1 μM) and Ca²⁺-dependent (Figure 1).

Effects of α₁- and α₂-adrenoceptor agonists

As shown in Figure 2, the α₂-adrenoceptor agonists, clonidine and oxymetazoline, tested at 0.01–1 μM, inhibited the evoked overflow of tritium in a concentration-dependent manner. The concentration-response curves for the two drugs were

Figure 2 Effects of clonidine (●), oxymetazoline (■), phenylephrine (□) or methoxamine (○) on the efflux of tritium evoked by electrical stimulation. Starting from $t = 75$ min, increasing concentrations of the drugs were added to the superfusion medium at intervals of 20 min. Points represent the mean $\pm$ s.e.mean (vertical bars) of 3–14 experiments in duplicate.

superimposable. The maximal effect was about 45% and the EC₅₀ was 0.03 μM. In contrast, the α₁-adrenoceptor agonists, phenylephrine or methoxamine, did not affect the overflow of tritium at concentrations up to 1 μM. At the concentrations used the drugs did not affect the spontaneous outflow of radioactivity.

Antagonism against clonidine

The α₂-adrenoceptor antagonist, yohimbine (0.01–0.1 μM), shifted the clonidine concentration-response curve increasingly to the right (Figure 3). The calculated pA₂ value was 8.29 (Schild slope = 0.98). In contrast, prazosin or AR-C 239, antagonists with clear preference for the α_{2B}- and the α_{2C}-adrenoceptor subtypes as opposed to the α_{2A} and α_{2D} subtype (Paris *et al.*, 1990; Blaxall *et al.*, 1991; Simonneaux *et al.*, 1991), did not change the effect of clonidine at 0.3 μM (Figure 4).

Mianserin antagonized the effect of clonidine in a stereoselective manner (Figure 5). The concentration-response curve of the agonist was shifted to the right in presence of 0.1 and 0.3 μM of (+)-mianserin (pA₂ = 7.74), whereas the (-)-enantiomer was ineffective at 0.3 μM. The novel selective α₂-adrenoceptor blocker ORG 20350 (Maura *et al.*, 1992) also antagonized clonidine (pA₂ = 7.55). The antagonists used did not affect the basal outflow of tritium.

Effects of α₂-adrenoceptor antagonists and of desipramine

Figure 6 illustrates changes of the electrically-evoked tritium overflow that were observed when slices of human brain were exposed to various α₂-adrenoceptor antagonists or to the NA reuptake inhibitor, desipramine. Yohimbine (0.1 μM) increased the evoked tritium overflow by about 30%. ORG 20350 and (+)-mianserin (0.1 and 0.3 μM), but not the (-)-enantiomer (0.3 μM), were also effective. Prazosin and AR-C 239 were ineffective at a concentration of 0.3 μM. Desipramine (1 μM) increased the overflow of tritium by about 40%. The effects of 0.1 μM yohimbine and 1 μM desipramine appeared to be additive.

Figure 3 Effect of yohimbine on the clonidine-induced inhibition of the electrically-evoked overflow of tritium. When used, yohimbine was present throughout the superfusion. Points represent the mean $\pm$ s.e.mean (vertical bars) of 3–4 experiments in duplicate: (●) clonidine; (○) clonidine + 0.01 μM yohimbine; (□) clonidine + 0.03 μM yohimbine; (Δ) clonidine + 0.1 μM yohimbine.

Figure 4 Effects of prazosin or AR-C 239 on the clonidine-induced inhibition of the electrically-evoked overflow of tritium. When used, prazosin or AR-C 239 was present throughout the superfusion. Means $\pm$ s.e.mean (vertical bars) of 3–5 experiments in duplicate are presented: (●) clonidine; (Δ) clonidine + 0.3 μ M prazosin; (□) clonidine + 0.3 μ M AR-C 239.

Figure 5 Effects of (-)-mianserin, (+)-mianserin or ORG 20350 on the clonidine-induced inhibition of the electrically-evoked overflow of tritium. When used, (-)-mianserin, (+)-mianserin or ORG 20350 was present throughout superfusion. Each point represents the mean $\pm$ s.e.mean (vertical bars) of 3–4 experiments in duplicate: (●) clonidine; (○) clonidine + 0.3 μ M (-)-mianserin; (■) clonidine + 0.1 μ M (+)-mianserin; (□) clonidine + 0.3 μ M (+)-mianserin; (▲) clonidine + 0.1 μ M ORG 20350; (Δ) clonidine + 0.3 μ M ORG 20350.

Interaction between clonidine and desipramine

The concentration-response curve of clonidine as an inhibitor of tritium overflow was shifted to the right when 1 μ M desipramine was present in the superfusion medium (Figure 7).

Discussion

The electrically-evoked tritium overflow from slices of human neocortical tissue prelabelled with [3 H]-NA was almost totally

Figure 6 Effects of desipramine and of α_2 -adrenoceptor antagonists on the electrically-evoked overflow of tritium. The effects of drugs were calculated by comparing the depolarization-evoked overflow at F_1 obtained in the presence of drugs and in standard medium (control conditions) as follows: $[(F_1\text{drug} - F_1\text{control})/F_1\text{control}] \times 100$. Means $\pm$ s.e.mean (vertical bars) of 3–5 experiments in duplicate are shown. Significance of changes versus the electrically-evoked overflow in standard medium was determined by Student's t test: * $P < 0.05$ and ** $P < 0.001$.

Figure 7 Effect of desipramine on the clonidine-induced inhibition of the electrically-evoked overflow of tritium. When used, desipramine was present throughout the superfusion. Points are mean $\pm$ s.e.mean (vertical bars) of 3 experiments in duplicate: (●) clonidine; (○) clonidine + 1 μ M desipramine.

Ca^{2+} -dependent and tetrodotoxin-sensitive, suggesting that the evoked overflow of radioactivity reflects a quasi-physiological, action-potential induced, exocytotic release of [3 H]-NA.

Studies in animals have shown that the overflow of NA produced by exposing brain slices of synaptosomes to different depolarizing stimuli can be modulated by autoreceptors classified as α_2 -adrenoceptors (see review by Starke, 1987). The results in Figure 2, showing that the electrically-evoked overflow of [3 H]-NA was inhibited by clonidine and oxymetazoline, two α_2 -adrenoceptor agonists, but not by the α_1 -adrenoceptor agonists, phenylephrine and methoxamine,

and the results of Figure 3, showing that the inhibitory effect of clonidine was attenuated by the α_2 -adrenoceptor antagonist, yohimbine, indicate that an α_2 -autoreceptor mechanism also exists in human brain.

Binding studies performed on various tissues and cell lines (reviewed by Docherty, 1989; see also Paris *et al.*, 1990; Blaxall *et al.*, 1991; Simonneaux *et al.*, 1991; Remaury & Paris, 1992) as well as rat and human receptor gene products (Kobilka *et al.*, 1987; Regan *et al.*, 1988; Lorenz *et al.*, 1990; Lanier *et al.*, 1991; Harrison *et al.*, 1991; Lomasney *et al.*, 1991) have led to a subdivision of α_2 -adrenoceptors into four pharmacologically distinct subtypes termed α_{2A} , α_{2B} , α_{2C} and α_{2D} .

According to the above studies, a number of drugs exist that are useful in discriminating between α_2 -adrenoceptor subtypes because their affinities for the subtypes differ by more than 10 fold. The antagonists which seem most selective are prazosin and AR-C 239 which display low to moderate affinity for the α_{2A} and α_{2D} subtypes but a high affinity for α_{2B} and α_{2C} sites. Conversely, the α_2 -adrenoceptor agonist, oxymetazoline, displays high affinity for α_{2A} and α_{2D} receptors and low affinity for the α_{2B} and α_{2C} subtypes. Our finding that oxymetazoline potentially inhibited the [3 H]-NA overflow is compatible with an involvement of α_{2A} or α_{2D} receptors. On the other hand, prazosin and AR-C 239 did not change the inhibitory effect of clonidine at 0.3 μ M, a concentration at least 30 fold higher than the K_i values obtained in binding studies (Bylund *et al.*, 1988; Michel *et al.*, 1989; Brown *et al.*, 1990; Lanier *et al.*, 1991; Simonneaux *et al.*, 1991; Uhlén & Wikberg, 1991; Remaury & Paris, 1992). Therefore the present results tend to exclude the involvement of receptors of the α_{2B} or α_{2C} subtype.

Using rat cerebral cortex synaptosomes we had found previously that the α_2 -autoreceptors were stereoselectively blocked by the (+)-enantiomer of mianserin, while the α_2 -adrenoceptors mediating inhibition of 5-hydroxytryptamine release were blocked with identical potency by the two enantiomers, suggesting the existence of α_2 -adrenoceptor subtypes (Raiteri *et al.*, 1983; Maura *et al.*, 1985). The human α_2 -autoreceptor also is sensitive to (+)-mianserin ($pA_2 = 7.74$), but not to the (-)-isomer used at 0.3 μ M (Figure 5).

Recently, it was reported that the affinity of (-)-mianserin for α_{2A} sites was 10–20 times higher than that for α_{2D} sites for which a pK_i value of 6.0 was found (Simonneaux *et al.*, 1991). These data, together with the poor sensitivity of the autoreceptors towards (-)-mianserin (Figure 5), suggest a classification of the autoreceptors as α_{2D} . However, more convincing experimental evidence is clearly necessary to discriminate between two receptor subtypes which appear to be pharmacologically very similar.

The pA_2 value for (+)-mianserin in the human brain (7.74) is close to that found in the rat (7.80; Maura *et al.*, 1985) suggesting similarity between the autoreceptors in the two species. The pA_2 of yohimbine in human cortex was 8.29. This value is almost superimposable on those reported for yohimbine tested against clonidine, in rat or rabbit brain (see Starke, 1987; Limberger *et al.*, 1991). The presynaptic α_2 -autoreceptors in rabbit or rat brain are known to be prazosin-insensitive (Hedler *et al.*, 1981; Reichenbacher *et al.*, 1982; Raiteri *et al.*, 1983; Gobbi *et al.*, 1990; Limberger *et al.*, 1991). Recently, the α_2 -autoreceptors in rat brain cortex were found to be insensitive also to AR-C 239 (Maura *et al.*, 1992). Finally, the novel α_2 -adrenoceptor blocker, ORG

20350, which blocked rat brain cortex autoreceptors ($pA_2 = 7.25$) but was ineffective at the α_2 -heteroreceptors regulating 5-hydroxytryptamine release (Maura *et al.*, 1992), blocked the human α_2 -autoreceptors with a pA_2 very close to that found in the rat. All together these data strongly suggest that the α_2 -autoreceptors in the brain of humans, rats and probably rabbits are pharmacologically very similar.

The results obtained when adrenoceptor antagonists were added to electrically-stimulated human cerebrocortical slices in the absence of exogenous agonist indicate that, under the stimulation conditions used, the autoreceptors were in part activated by NA endogenously released and present in the receptor biophase (see Limberger *et al.*, 1989): yohimbine (but not prazosin or AR-C 239), (+)-mianserin (but not the (-)-enantiomer) and ORG 20350 increased the evoked [3 H]-NA overflow. These data (a) are in keeping with the receptor subclassification proposed above; (b) suggest that, under certain conditions, the autoreceptors can be activated by the synaptically released NA; (c) are compatible with the view that in human cerebral cortex, clonidine acts where endogenous NA acts.

The data of Figure 6 show, probably for the first time in man, that two antidepressants, desipramine and mianserin (actually its (+)-enantiomer) can enhance NA overflow, the former by preventing NA reuptake and the latter by preventing autoreceptor activation due to endogenous NA. The (apparent) additivity of the effects of desipramine and of yohimbine reflects the different mechanisms involved.

As shown in Figure 7, the presence of desipramine largely prevented the effect of clonidine on [3 H]-NA overflow. It has long been known that tricyclic antidepressants may inhibit the centrally-mediated antihypertensive effect of clonidine, although the mechanism of this interaction has not been clarified (Gerber & Nies, 1990). Our results with human brain tissue are compatible with the view that clonidine loses activity because it has to compete for α_2 -adrenoceptors (either pre- or postsynaptically located) with endogenously released NA, the concentration of which in the receptor biophase is raised following reuptake inhibition by desipramine. This interpretation is in line with the previous finding that the interaction between clonidine and desipramine does not occur in superfused synaptosomes, i.e. in conditions in which the concentration of the released NA can not increase in the vicinity of the receptors (Maura *et al.*, 1984).

In conclusion, terminal α_2 -autoreceptors which may be involved in a local negative feedback regulation of NA release exist in human cerebral cortex. The pharmacological characterization suggests that these autoreceptors are not α_{2B} or α_{2C} . According to the present classification, they belong either to the α_{2A} or to the α_{2D} subtype. Data obtained with a number of drugs, including the enantiomers of mianserin and ORG 20350, show that the noradrenaline autoreceptors in human and rat brain are pharmacologically very similar. Thus the rat brain is likely to represent a useful model to characterize better terminal noradrenaline autoreceptors in the central nervous system and to develop more selective α_2 -adrenoceptor drugs.

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