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Specific Binding of Nicergoline on an α_1-like Adrenoreceptor in the Rat Retina

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Abstract

Systemic treatment with nicergoline, an ergoline derivative showing α_1-antagonist properties, causes vasodilatation in the eye without apparent untoward cardiovascular effects. In the present work we investigated the ability of nicergoline to inhibit the binding of radiolabelled prazosin in the rat retina and cortex.

We found that nicergoline inhibited [^{3}H]prazosin binding in both tissues, being more potent than unlabelled prazosin in the retinal tissue. The competition curves of the ergoline derivative were well fitted by a one-site model in the cortical tissue, with an IC50 (concentration of the drugs needed to inhibit the binding of labelled prazosin by 50%) of $2{\cdot}54 \times 10^{-8}$ M, and by a two-site model in the retinal tissue, with IC50 values of $7{\cdot}08 \times 10^{-12}$ M and $1{\cdot}82 \times 10^{-5}$ M. 2-(2,6 dimetoxyphenoxyethyl) aminomethyl-1,4-benzodioxane hydrochloride (WB4101) and phentolamine, selective ligands for the high-affinity binding site for prazosin, in particular the α_{1A}-site, fully inhibited prazosin binding in the cortex but only partially inhibited prazosin binding in the retina, being less potent in this tissue than either nicergoline or prazosin.

Our results suggest that a binding component of α_1-adrenoreceptors is expressed to a lesser extent in the retina than the cortex, leading to a reduced response of the retinal tissue to prazosin, and more particularly to WB4101 and phentolamine. The selective binding of the nicergoline on this retinal adrenoreceptor may explain the peculiar efficacy of the drug in ocular pathophysiology.

Pharmacological studies on ergot alkaloids and their derivatives have shown them to have a variety of actions on the central and peripheral nervous systems. The ergoline nucleus contained in ergot alkaloids is also a common feature of the three monoamine transmitters dopamine, noradrenaline and serotonin. This structural relationship may explain the affinity of ergot derivatives for specific monoaminergic receptors (Berde & Schild 1978).

Nicergoline, 8b-(5-bromonicotinoylhydroxy-methyl)-1,6-dimethyl-10a-methoxyergoline, is an ergoline derivative whose pharmacological effects resemble those of prazosin, a non selective α_1-adrenoreceptor antagonist; indeed nicergoline shows vasodilating and α-adrenoreceptor-blocking activity (Arcamone et al 1972). In contrast to prazosin, nicergoline crosses the blood–brain barrier and acts on the central nervous system (CNS) (Chelly & Huguet 1981). Biochemical binding studies have confirmed that nicergoline has a high affinity for α_1-adrenergic receptors (Huguet et al 1980) and for this reason it is proposed as a vasodilator in the treatment of cerebral ischaemia (Moretti et al 1985).

Nicergoline's actions in the CNS lead to stimulation of catecholamine turnover, particularly that of dopamine, in the mesolimbic system (Moretti et al 1988) and also of phosphatidyl inositol turnover, crucial for the process of learning and memory (Fariello & Carfagna 1991). Innovative treatments with nicergoline were therefore proposed which combinined the nootropic effects of the drug with mental exercise, for the management of pathological mental disorders (Elwan et al 1995).

In the peripheral regions the effects of nicergoline appear to be limited to the eye. It has been

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reported that systemic treatment with nicergoline causes vasodilatation in human eyes without producing general hypotension or affecting the cardiac rhythm (Bec et al 1976) and nicergoline has been suggested for the treatment of glaucoma to reduce intra-ocular pressure (Reibaldi et al 1985).

However, to date not much is known about the molecular mechanisms by which nicergoline affects retinal receptors, and in particular α_1-adrenoreceptors. It is known that subtypes of α_1-adrenoreceptors are expressed in several tissues. These can be classified pharmacologically on the basis of their sensitivity to several antagonists. For example, 2-(2,6-dimethylphenoxyethylamino methyl-1-4-benzodioxone hydrochloride (WB4101) binds with high affinity to the α_{1B}-adrenoreceptor subtype and with low affinity to the α_{1A}-adrenoreceptor subtype.

In the present study we determined the biochemical binding characteristics of nicergoline on α_1-adrenoreceptors in rat retinal synaptosomal membranes and in the rat cortex. Competition experiments were performed with nicergoline, phentolamine and WB4101 in the presence of 2 nM [^{3}H]prazosin which, rather unselectively, labels most of the α_1-adrenoreceptor subtypes.

Materials and Methods

Male Wistar rats (280–300 g) were killed by decapitation. The cerebral cortex was carefully dissected. Eyes were enucleated and the globe was opened longitudinally. After removing the lens, the retina was deducted with curved-tip forceps. After isolation, the tissues were immediately frozen in liquid nitrogen and stored at −80°C.

Binding study

Membrane preparation. The cortex tissue and retina were homogenized with a Polytron homogenizer (setting 15; three times each of 30 s) in a solution (volumes of 20–60 mL) containing TRIS–HCl buffer (50 mM; pH 7·4) and 0·1 mM phenylmethylsulphonylfluoride (PMSF). The tissue homogenates were centrifuged for 15 min at 1000 *g*; the pellet was discarded and the supernatant was centrifuged again at 30 000 *g* for 30 min for the cortex and 10 min for the retina. The resulting pellets were suspended again with ice-cold potter glass–teflon in the incubation buffer (50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0·1% ascorbic acid; pH 7·4) to obtain 350 μg of protein in 400 μL homogenate. The suspensions were filtered through a double-layer of cheese-cloth. Each tube contained 400 μL of homogenate and a constant amount of labelled prazosin (final volume of 1 mL).

Binding assays. Inhibition experiments were performed in the presence of 2 nM [^{3}H]prazosin (specific activity 77·2 Ci mmol^{-1} Dupont-Hen, Boston, MA), and nonspecific binding was defined by 1 μM prazosin. The reaction was initiated by the addition of homogenate and the mixture was incubated for 50 min at 25°C. The reaction was terminated by addition of ice-cold 50 mM Tris–HCl buffer followed by three washes with the same buffer under vacuum filtration over Whatman GF/B filters. The filters were removed and placed in scintillation vials, solubilised with 7 mL of Pico-Aqua scintillation fluid (Packard) and left overnight before counting on a Beckman LS 6000IC scintillation counter. The results were analysed using the computer program LIGAND (Munson & Robard 1980) and the specific binding was subjected to transformation for estimation of B_{max} and K_d. Inhibition studies were performed with a single concentration (2 nM) of [^{3}H]prazosin in the presence of increasing concentrations of competing ligands: prazosin, WB4101, nicergoline and phentolamine. In the range of concentrations tested, ethanol did not interfere with the binding assay (solvent control).

Protein measurement was carried out by the method of Bradford (1976), using bovine serum albumin (BSA) as protein standard.

Data analysis

All the results are presented as mean ± s.d. of four experiments. The concentration–response relationships could be fitted using an algorithm based on the least-squares fitting routine in the case of monophasic behaviour (equation 1):

$$\% \text{ Inhibition} = 1/(1 + (\text{IC50}/[\text{Drug}])^{n}) \quad (1)$$

or by the sum of two terms in the case of biphasic behaviour (equation 2):

$$\% \text{ Inhibition} = 1/(1 + (\text{IC50}_1/[\text{Drug}])^{n1}) + 1/(1 + (\text{IC50}_2/[\text{Drug}])^{n2}) \quad (2)$$

where % inhibition is the differences between the total binding and the non-specific binding normalized to the maximal inhibition measured; IC50 is the concentration of the drugs needed to inhibit the binding of labelled prazosin by 50%; n is the slope factor of the curves; and [Drug] is the drug concentrations. The goodness of the fit of the function to the experimental data was evaluated by computing the coefficient of determination (R), the standard deviation of the function (σ) and $F_{0.95}$ that came from the analysis of variance (Tallarida & Murray 1986). The R and σ values were calculated by equations 3 and 4:

$$R = (1 - SSE/SS) \quad (3)$$

$$\sigma^2 = \sum(exp - theor)^2/N - 1 \quad (4)$$

where SSE is the sum of the squared errors; SS is the sum of the squares of the data; exp is the experimental value; theor is the calculated value; and N is the number of data. The selection of a one-site or two-site model to fit the data was based on the calculated values of R and σ, and on the values of $F_{0.95}$ (Tallarida & Murray 1986). For instance, the closer that the value of R was to 1 and the lower the value of σ, the better the fit. Further, if the computed value of $F_{0.95}$ was less than the tabular value, the means did not differ significantly (Tallarida & Murray 1986).

The dissociation constant for the competitor (K_I) was calculated according to the Cheng & Prusoff (1973) transformation taking into account the IC50 of the drug, the K_d and the concentration of radioligand used.

Drugs

Nicergoline was from SIFI S.p.a. (Lavinaio, CT, Italy). Prazosin and phentolamine were purchased from Sigma (St Louis, MO, US). WB4101 was purchased from RBI (Natik, MA). All the other reagents were of analytical grade. The experiments with prazosin were carried out in the dark (sodium lamp) to prevent photodegradation.

Results

Under equilibrium conditions, the binding of [^{3}H]prazosin to retinal membranes increased with increasing amounts of added protein and was linear within the concentration range 150–400 μg of protein per tube.

Competition curves were therefore constructed by using unlabelled prazosin, nicergoline, WB4101 and phentolamine against the [^{3}H]prazosin (2 nM). The cortical synaptosomes binding-experiments showed that the homologous inhibition of [^{3}H]prazosin was well fitted with the function based on a one-site model but with a slope factor (n) lower than unity; the F-test did not show significant differences between the fitting of the data using a one-site model and two site-model. The IC50 calculated by fitting routine was $2{\cdot}39 \times 10^{-10}$ M (n = 0·56) (R = 0·99, σ = 6·57) (B_{max}: 290 fmol (mg protein)$^{-1}$) (Figure 1).

In the same tissue, the competition curves of nicergoline, WB4101 and phentolamine were also fitted with the function based on a one-site model but showing corresponding n values lower than unity. For example, the IC50 of nicergoline, WB4101 and phentolamine were $2{\cdot}54 \times 10^{-8}$ M (n = 0·62) (R = 0·96, σ = 5·56), $1{\cdot}06 \times 10^{-8}$ M (n = 0·55) (R = 1, σ = 4·58) and $8{\cdot}07 \times 10^{-7}$ M (n = 0·68) (R = 0·93, σ = 7·58), respectively (Figure 1). The corresponding K_I values calculated by Cheng & Prusoff (1973) transformation were $7{\cdot}26 \times 10^{-9}$ M, $3{\cdot}03 \times 10^{-9}$ M and $2{\cdot}31 \times 10^{-7}$ M.

In the retinal membranes the homologous inhibition of [^{3}H]prazosin followed a biphasic behaviour as supported by the fact that the F-test showed significant differences between the fitting of the data using a one-site model and two site-model (Figure 2). The IC50 values calculated by fitting routine were $5{\cdot}59 \times 10^{-11}$ M (n = 0·83) and $1{\cdot}28 \times 10^{-5}$ M (n = 0·81) (R = 0·99, σ = 6·57) (B_{max}: 603 fmol (mg protein)$^{-1}$).

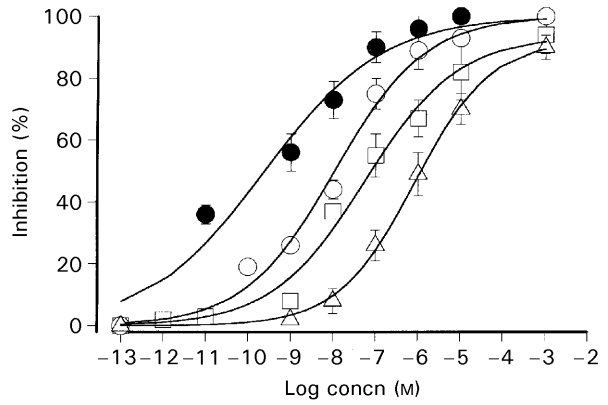

Figure 1. Inhibition curves of prazosin ●, WB4101 ○, nicergoline □ and phentolamine △ on rat cortical membranes. Each point represents the mean ± s.d. of 9 experiments (s.d. is in 10% range). Rat cortex membranes were incubated at 25°C for 30 min with [^{3}H]prazosin (2 nM) in the presence of the different drugs (10^{-13}–10^{-3} M).

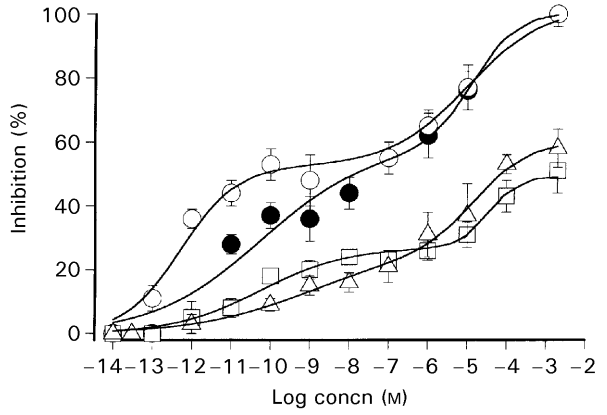

Figure 2. Inhibition curves of prazosin ●, WB4101 ○, nicergoline □ and phentolamine △ on rat retinal membranes. Each point represents the mean ± s.d. of 7 experiments (s.d. in 10% range). Rat retinal membranes were incubated at 25°C for 30 min with [^{3}H]prazosin (2 nM) in the presence of the different drugs (10^{-14}–10^{-2} M).

In the same tissue, the inhibition curve of nicergoline was also described by a two-site model (F-test showed significant differences between the fitting of the data using a one-site model and two site-model), nicergoline being more potent than unlabelled prazosin within the concentration range 10^{-14}–10^{-8} M (Figure 2). The IC50 and K_I values of nicergoline calculated, for this concentration range, by fitting routine were $7{\cdot}08 \times 10^{-12}$ M (n = 0·82) (R = 0·96, $\sigma = 3{\cdot}47$) and $2{\cdot}66 \times 10^{-12}$ M, respectively. No significant differences were observed between nicergoline and prazosin inhibition curves within the concentration range 10^{-6}–10^{-3} M. The corresponding IC50 and K_I values of nicergoline were $1{\cdot}82 \times 10^{-5}$ M (n = 0·8) and $6{\cdot}84 \times 10^{-5}$ M, respectively.

In the retinal membrane, WB4101 and phentolamine only partly displaced the [^{3}H]prazosin (Figure 2). The IC50 of WB4101 and phentolamine, calculated for the concentration range 10^{-14}–10^{-8} M by fitting the data using the two-site model function, were $5{\cdot}63 \times 10^{-10}$ M (n = 0·41) and $9{\cdot}29 \times 10^{-10}$ M (n = 0·43), respectively. Also in these cases the use of the two-site model function resulted in a better resolution than that obtained using the one-site model, as determined by F-test. The K_I of WB4101 and phentolamine calculated by Cheng & Prusoff (1973) transformation were $2{\cdot}12 \times 10^{-10}$ M and $3{\cdot}49 \times 10^{-10}$ M, respectively. It was not possible to accurately calculate the fitting parameters at high concentrations because neither drug fully displaced the [^{3}H]prazosin from the receptors.

Discussion

We demonstrated here that nicergoline, which is an α_1-adrenoreceptor antagonist in the cortex, binds also to the α_1-adrenoreceptor in the rat retina. However, a significant difference appears to exist in the responses of the cortex and retina to nicergoline binding. This is demonstrated by the fact that in the cortex nicergoline fully inhibited the binding of labelled prazosin following a monophasic pattern whereas in the retina the drug again inhibited the binding of the labelled α_1-antagonist, but with a biphasic behaviour. This can be explained by differences in the receptor populations expressed in the retina and in the cortex.

It is known that the cortex contains at least two types of α_1-adrenoreceptors, often called low- and high-affinity binding sites for prazosin, that can be distinguished on the basis of their molecular structure and pharmacological properties (Oshita et al 1991; Michel & Goepel 1998). The high-affinity site can be further classified into the α_{1A}- and α_{1B}-subtypes that are labelled, respectively, with high and low affinity, by both WB4101 and phentolamine (Oshita et al 1991; Michel & Goepel 1998). Prazosin, however, is not capable of distinguishing between α_1-adrenoreceptor subtypes. Therefore, in our experiments it appears that one binding component of α_1-adrenoreceptor subtypes which is present in the rat cortex seems to be expressed less in the retinal tissue. We believe that this component is represented by the low-affinity α_1-adrenoreceptor site for prazosin. We come to this conclusion considering that the WB4101, a well known selective α_{1A}-antagonist (Salles & Badia 1994), fully inhibited binding of labelled prazosin in the cortex but did not exert a similar effect in the retina. Similarly, phentolamine, an α_1-antagonist selective for the high-affinity sites (Oshita et al 1991; Michel & Goepel 1998), also inhibited the binding of labelled prazosin in the cortex but almost failed to exert a similar effect in the retina. The lower expression of one binding component, possibly the low-affinity α_1-adrenoreceptor site, in the retina and the subsequent predominance of at least one other α_1-adrenoreceptor population is also supported by the fact that the two-site model function describes the experimental data of all the drugs used including prazosin.

Multiple receptor sites for prazosin, possibly low- and high-affinity sites, are normally expressed in the rat cortex, as demonstrated by the slope factors of the inhibition curves of the drugs studied being lower than unity. The existence of pharmacologically distinct α_1-adrenoreceptor subtypes has also been proposed in other tissues (Hiramatsu et al 1994; Wada et al 1996; O'Malley et al 1998). Differences in the receptor population ratio α_{1A}/α_{1B} in the retina and in the cortex may also explain the different sensitivity showed by the adrenoreceptors to drugs under study in the two tissues.

Interestingly, it was observed that nicergoline was more potent than the well known α_1-adrenoreceptor antagonists prazosin and phentolamine in inhibiting binding of labelled compound in the retina. These findings open the question of whether this drug can replace α_1-adrenoceptor antagonists such as prazosin and phentolamine in the treatment of several eye disorders. The effects of the classical α_1-adrenoceptor antagonists are not limited to the eyes. For example, α_1-adrenoreceptor antagonists, even after ocular administration, cause vasodilatation and cardiac abnormalities (Smith et al 1979) that have not been observed with nicergoline treatment. Therefore, the use of low concentrations of a more selective retinal adrenoreceptor antago-

nist such as nicergoline would limit the appearance of unwanted side effects, particularly during chronic therapies of ocular disorders.

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