International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 1 (4): 299-310, 2005
ISSN 1811-7775

Does the Binding Duration of a Partial α_2-adrenoceptor Agonist Exceed its Activation Interval at the Autoreceptor?

[1]Marc Steffens, [1]Anna Olayioye, [1]Boris Huber, [2]Clemens Allgaier and [1]Thomas J. Feuerstein
[1]Sektion Klinische Neuropharmakologie der Neurochirurgischen Universitätsklinik, Neurozentrum, Breisacherstr. 64, D-79106 Freiburg, Germany
[2]Institut für Pharmakologie und Toxikologie, Universität Leipzig, D-04107 Leipzig, Germany

Abstract: A ligand may be an agonist, activating the receptor, or an antagonist, blocking the receptor, or both, i.e. a partial (ant)agonist. Mechanistically, partial agonism could to be due to a binding interval longer than necessary for receptor activation. Then, the partial agonist would act as a full agonist during the activation interval and as a full antagonist thereafter. To test this hypothesis, the effects of different temperatures were evaluated in studies on electrically evoked [^{3}H]-noradrenaline ([^{3}H]-NA) release and in binding experiments at (predominantly presynaptic) α_2-adrenoceptor sites, using rat neocortex and hippocampus slices and synaptosomes. The α_2-autoreceptor-mediated inhibition of [^{3}H]-NA release by a full (UK14304) and two partial (clonidine, guanfacine) agonists was tested in the absence and presence of autoinhibition. In addition, blockade of autoinhibition, i.e. the inhibitory effect of the endogenous full agonist NA, was analysed at different temperatures. In corresponding experiments, the binding to α_2-adrenoceptor sites of [^{3}H]-NA and the inhibition of this binding by UK14304, clonidine and guanfacine were evaluated. Concentration-inhibition curves of UK14304, clonidine and guanfacine in the absence of autoinhibition at 17 to 42°C revealed estimates of the concentrations of half maximum inhibition (IC_{50}) and of the maximum inhibitions (I_{max}). IC_{50} and I_{max} values of UK14304 were not dependent on the temperatures applied. In contrast, the corresponding values of clonidine and guanfacine changed with temperature. At low temperature the partial agonists enhanced their antagonist properties. This was confirmed by the complete loss of the inhibitory effect of clonidine at 22°C in the presence of autoinhibition, i.e. when endogenous NA competed with the exogeneous ligand for the α_2-autoreceptors. At 37°C, however, clonidine displayed agonist properties also in the presence of endogenous NA as UK14304 did at both 22 and 37°C. The disinhibition of [^{3}H]-NA release, i.e. the blockade of autoinhibition, in the presence of the pure α_2-adrenoceptor antagonists, idazoxan and rauwolscine, was the same at 22 and 37°C. The different influence of changing the temperature in the case of a full and of a partial agonist was confirmed in saturation and competition binding studies: Saturation binding K_ds of the full agonist [^{3}H]-NA were equivalent at high and low temperature; the K_i estimates of UK14304 were also independent of the temperatures applied, whereas K_i values of clonidine and guanfacine increased with lower temperatures. It is concluded that a full agonist is characterised by a short binding phase, just sufficient for receptor activation and smaller than the whole interval during which the receptor can be activated. The binding duration of a partial agonist, however, exceeds its activation interval at the receptor. Temperature reduction increases this excess of binding duration relative to the activation interval of a partial agonist.

Key words: α_2-autoreceptor sites, partial (ant)agonist, UK14304, clonidine, guanfacine, [^{3}H]-noradrenaline release, binding studies

INTRODUCTION

Concentration-response curves of full agonists and of partial agonists differ in their maximum effects in systems without spare receptors. An example is the α_2-autoreceptor-mediated inhibition of evoked [^{3}H]-noradrenaline ([^{3}H]-NA) release in rat hippocampal slices caused by UK14304, a full agonist and clonidine, a partial agonist[1,2]. May a difference in the receptor interactions of these two ligands, i.e. association to and dissociation from, α_2-autoreceptors explain their different efficacies? It was the aim of the present study to give a

Corresponding Author: Dr. Thomas J. Feuerstein, Sektion Klinische Neuropharmakologie der Neurochirurgischen Universitätsklinik, Neurozentrum, Breisacherstr. 64, D-79106 Freiburg, Germany
Tel: ++761 270 5280 Fax.: ++761 270 5281 E-mail: thomas.feuerstein@uniklinik-freiburg.de

simple answer to this question by a simple experimental approach, despite highly sophisticated attempts in the literature to explain partial agonism over decades[3,4]. The present study, for instance, disputes the statement that activation must affect binding since binding affects activation[5]. Our experimentally founded doubts about this statement refer to the finding that the different efficacies of the full agonist UK14304 and the partial agonists clonidine and guanfacine[6] can sufficiently be explained on the level of binding and have not necessarily to involve steps of the signal transduction chain, subsequent to binding.

Some elementary experiments are described in the following. These experiments utilise the fact that changes in temperature affect the kinetics of ligand-receptor interactions. Present results suggest the following straightforward explanation of the difference between partial and full agonists, being valid at least for the chosen example of inhibition of [^{3}H]-NA release through α_2-autoreceptors activated by NA itself, by UK14304, clonidine and guanfacine: Partial - as compared to full - agonism is seen when the binding duration between the agonist molecule and the receptor protein exceeds the time required for receptor activation. After activation, the full agonist diffuses immediatly from the receptor and another agonist molecule may affix and activate the receptor. The partial agonist molecule, however, remains bound for longer than necessary for activation, thereby preventing the binding of another partial agonist molecule to the receptor. In other words, after its initial activation the partial agonist behaves as pure competitive antagonist.

MATERIALS AND METHODS

Superfusion experiments: Neocortical and hippocampal tissue was obtained from male Wistar rats weighing 250-350 g. The principles of laboratory animal care were followed in accordance with the declaration of Helsinki. Slices (thickness: 350 μm) of cortical tissue were washed three times in 5 ml cold buffer and then incubated at 37°C in 2 mL of buffer containing 0.1 μM (-)-[^{3}H]-NA for 30 min. Neocortical slices were then superfused in parallel with medium at 0.4 mL min^{-1} at 17, 37 and at 42°C. Hippocampal slices were correspondingly superfused at 22°C and at 37°C. The incubation and superfusion medium contained (mM): NaCl 118, KCl 1.8, $CaCl_2$ 1.3, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11 and ascorbic acid 0.57. The superfusion medium of hippocampal slices contained (+)-oxaprotiline (1 μM) additionally. Desipramine (1 μM) was present in the superfusion fluid when guanfacine (see below) was tested in neocortical slices at 17 and 37°C.

Four periods of electrical stimulation were applied consisting of rectangular pulses of 2 ms width and a current strength of 76 mA (necortex slices) or 32 mA (hippocampal slices). The stimulations (S_1 to S_4) occurred at t = 75, 110, 145 and 180 min during superfusion. Release conditions free of autoinhibition were obtained with 4 pulses, 100 Hz (pseudo-one-pulse conditions[7,8]. All exogenous α_2-adrenoceptor agonists were tested in autoinhibition-free conditions.

An autoinhibitory tone through α_2-autoreceptors was achieved when neocortex slices were subjected to S_1-S_4 stimulations with 360 pulses of 2 ms width, at 3 Hz[7]. In these experiments, (+)-oxaprotiline (1 μM) was either absent (current strength then 76 mA, experiments with the α_2-adrenoceptor antagonists idazoxan and rauwolscine, below) or present (current strength 32 mA, experiments in hippocampal slices with the α_2-adrenoceptor agonists UK14304 and clonidine) throughout superfusion. When guanfacine was tested under autoinhibition-free conditions in neocortex slices desipramine (1 μM) was present and the current strength was 76 mA.

The first 75 min of superfusion served to remove excess radioactivity. The superfusion fluid was not collected during this period. Thereafter, 5 min fractions of the superfusate were collected continuously. The autoreceptor agonists UK14304, clonidine and guanfacine or the antagonists idazoxan and rauwolscine were added (at increasing concentrations in the case of concentration-response experiments) 15 min before S_2, S_3 and S_4. Mostly, the drugs to be compared were applied in the same experiments in order to improve the comparability of their effects. The outflow of tritium was calculated as a fraction of the tritium content of the tissue at the beginning of the respective collection period and expressed as fractional rate (per min). The electrically evoked overflow of tritium was calculated by subtracting the estimated basal outflow from the total tritium outflow during the collection period in which the stimulation period was applied plus the three periods thereafter; the basal outflow of tritium was assumed to decline linearly from the collection period before to the collection period 20 min after onset of stimulation. The overflow of tritium was then expressed as a percentage of the tritium content of the slice at the time of stimulation. For further evaluation of the stimulation-evoked overflow of tritium, ratios were calculated of the overflow evoked by S_2, S_3 and S_4 and the overflow evoked by S_1 (S_2/S_1, S_3/S_1 and S_4/S_1). Moreover, effects of the α_2-autoreceptor ligands on the stimulation-evoked overflow were calculated, for each single slice, as percentage effect, using the corresponding mean average control S_2/S_1, S_3/S_1 and S_4/S_1 ratios (solvent-treated slices, no agonist, no antagonist) as a reference.

Preparation of synaptosomes: For binding experiments, neocortical and hippocampal tissue samples were stored at -80°C until use. Synaptosomes were then prepared freshly for each experiment in order to enrich presynaptic binding sites. Thawed brain samples (0.3-0.5 g) were homogenised in 10 volumes (w/v) of ice cold sucrose (0.32 M)/HEPES (2.5 mM) buffer, pH 7.4. The following centrifugation steps were carried out in a Heraeus Biofuge 28RS (Osterode, Germany) at 4°C. The initial homogenate was centrifuged at 1000 x g for 10 min. The resulting supernatant was separated and centrifuged again at 10000xg for 10 min. The supernatant was discarded and the pellet was stored on ice until further use.

Saturation binding experiments: The procedure corresponded to that described by U´Prichard and Snyder[9]. Various concentrations of NA (0.32-320 nM) were used in order to determine saturation characteristics. For this purpose, specific activity of [^{3}H]-NA was decreased by adding different concentrations of unlabelled NA. Pellets were resuspended in ice-cold binding buffer, composed of (in mM): Tris-HCl 50, $MgCl_2$ 3, EDTA 1, ascorbic acid 0.6, pargyline 0.005, pH 7.4 and incubated for 15 min at 22°C to obtain complete MAO inhibition in the synaptosomal preparation. The binding reaction was initiated by adding 100 µL synaptosomal preparation (corresponding to a protein content of approximately 250 µg) to 880 µL assay-buffer (composition see above), 10 µL [^{3}H]-NA and 10 µL unlabelled NA, followed by a 30 min incubation at 22 or at 37°C. Specific binding was defined as total binding minus binding in the presence of 10 µM idazoxan and was determined for each concentration of [^{3}H]-NA/NA.

Reactions were finished by rapid filtration through Whatman GF/C glass fiber filters soaked in buffer containing 0.1% polyethylenimine (PEI) using a 96 well harvester (Brandel M96, Gaithersburg, MD, USA). The filters were washed with 3 mL of ice-cold assay buffer and transferred into scintillation vials. After addition of 3 mL Ultima Gold liquid scintillation cocktail (Packard Bioscience, Groningen, Netherlands) the filters were shaken thoroughly for 1 h. Radioactivity in the filters was determined using a Tri-Carb 2100TR liquid scintillation analyzer (Packard Instruments, Meriden, CT, USA).

Competition binding experiments: The procedure corresponded also to that described by U´Prichord and Snyder[9]. Pellets were resuspended in ice-cold binding buffer, as described above and incubated for 15 min at 22°C. The binding reaction was started after addtion of 100 µL synaptosomal suspension (corresponding to a protein content of approximately 250 µg) to 900 µL of binding buffer containing 5 nM [^{3}H]-NA and various concentations of the competing drug. After a 60 min incubation at different temperatures (17, 22, 37 and 42°C) the reaction was terminated by rapid filtration through Whatman GF/C filters (soaked in 0.1% polyethyleneimine) using a 96 well harvester (Brandel M96, Gaithersburg, MD, USA). The filters were washed with 3 mL of ice cold buffer and transferred into scintillation vials. After addition of 3 mL Ultima Gold liquid scintillation cocktail the filters were shaken thoroughly for 1 h. Radioactivity in the filters was determined using a liquid scintillation analyzer.

Specific binding of [^{3}H]-NA was defined as total binding minus binding in the presence of 10 µM idazoxan. In neocortical synaptosomes specific binding amounted to 52.85% of total binding, 95% confidence interval (CI_{95}) [50.90 and 54.85%], n = 49, at 17°C, to 41.26%, [38.72 and 43.80%], n = 46, at 37°C and to 3.70%, [-1.25 and 8.65%], n = 35, at 42°C. In hippocampal synaptosomes, specific binding amounted to 44.24%, [40.52 and 47.97%], n = 43, at 22°C and to 38.30%, [36.66 and 39.94%], n = 42, of total binding at 37°C.

Data analysis: A logistic function, representing the (semi-logarithmic) log-concentration-response relationship,

$$B = B_{max} \frac{10^{lg[NA]c}}{10^{-pK_dc} + 10^{lg[NA]c}} \quad (1)$$

with B being the specific binding in pmol/(mg protein) (dependent variable) and lg[NA] = $\log_{10}$ of the concentration (M) of [^{3}H]-NA/NA (independent variable) was applied to evaluate saturation binding experiments. The parameters to be estimated were B_{max}, the asymptotic maximum of binding, i.e. the number of binding sites per mg protein and pK_d, the negative $\log_{10}$ of the dissociation constant K_d between NA and the α_2-adrenoceptor. The estimate of the slope factor *c* served to decide whether a bimolecular reaction between NA and its binding site occurred[2]; *c* corresponds to the Hill coefficient). Thus, an estimate of *c* near unity with a sufficiently narrow CI_{95} allowed the assumption of a bimolecular reaction and a single K_d.

The following logistic function was used to evaluate data obtained from competition binding experiments (2a) and from superfusion experiments (2b):

$$B_{norm} = 1 - \frac{I_{max} 10^{lg[inhibitor]c}}{10^{-pIC_{50}c} + 10^{lg[inhibitor]c}} \quad (2a)$$

$$\frac{S_x}{S_1} = 1 - \frac{I_{max} 10^{lg[inhibitor]c}}{10^{-pIC_{50}c} + 10^{lg[inhibitor]c}} \quad (2b)$$

with B_{norm} being normalized binding, S_X/S_1 being normalized [^{3}H]-NA release (dependent variable) and lg[inhibitor] = $\log_{10}$ of the concentration (M, independent variable) of clonidine, guanfacine or UK14304, respectively, to be evaluated with respect to an inhibitory property on [^{3}H]-NA binding or on [^{3}H]-NA release, respectively. The parameters to be estimated were I_{max}, the asymptotic maximum of relative inhibition [0, ..., 1] and pIC_{50}, the negative $\log_{10}$ of the inhibitor concentration leading to half-maximum inhibition. Again, the estimate of the slope factor *c* served to decide whether a bimolecular reaction between α_2-adrenoceptor and inhibitor occurred. In the case of competition binding data with a *c* being near unity another evaluation could take place which yielded an estimate of the dissociation constant between receptor and inhibitor, K_i, considering the (small) difference between the descriptive IC_{50} value and K_i, the latter mechanistically interpretable as dissociation constant. Instead of applying the Cheng-Prusoff conversion of IC_{50} to K_i ($IC_{50} = K_i(1 + [NA]/K_d) = K_i+[NA]K_i/K_d$, with [NA] = [substrate][10], we preferred to introduce the concentration of the ligand [^{3}H]-NA directly into the function to be evaluated by nonlinear regression analysis (conversion of function (2a) with c = 1 into function (3)):

$$B_{norm} = 1 - \frac{I_{max}\ 10^{lg[inhibitor]}}{10^{-pK_i} \pm 10^{-lg[NA]-pK_i+pK_d} + 10^{lg[inhibitor]}} \quad (3)$$

B_{norm} was again normalized binding (dependent variable) and lg[inhibitor] = $\log_{10}$ of the concentration (M, independent variable); lg[NA] was $\log_{10}$ of the applied concentration of [^{3}H]-NA (5 nM = $10^{-8.30}$ M). This concentration of [^{3}H]-NA was adjusted by the ratio K_i/K_d (= $10^{-pKi+pKd}$, since $pK_i = -lgK_i$ and $pK_d = -lgK_d$) in order to account for different affinities of the receptor to [^{3}H]-NA and to clonidine, guanfacine or UK14304, respectively. In this case, K_d, or pK_d was taken from the estimation using the above mentioned function (1). The advantage of this procedure was that an estimate of the CI_{95} of pK_i was obtained directly.

Such a consideration of the competition for the same (auto)receptor of [^{3}H]-NA and of clonidine, guanfacine or UK14304, respectively, was not necessary in the case of superfusion experiments with pseudo-one-pulse conditions (see above) since these conditions guaranteed evoked [^{3}H]-NA release free of autoinhibition. Then, with a *c* near unity the IC_{50} value of clonidine, guanfacine or UK14304 corresponded to the K_d of the respective inhibitor.

Means of results and the parameters from the fitting procedures are given as estimates with CI_{95} (NLIN) of the SAS system; SAS Institute, Heidelberg, Germany) to indicate statistical significance[11]. Thus, significant differences between parameters with non-overlapping CI_{95} may be assumed.

Drugs: Purchased drugs were (-)-[ring-2,5,6-^{3}H]-NA, specific activity 40-80 Ci/mmol (PerkinElmer, Rodgau, Germany); (+)-oxaprotiline (Novartis, Basel, Switzerland); 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline tartrate (UK14304; Pfizer, Sandwich, Kent, UK); clonidine HCl (Boehringer, Ingelheim, Germany); idazoxan HCl, rauwolscine HCl, (Research Biochemicals International, Natick, USA.); desipramine HCl, guanfacine HCl (Sigma, Taufkirchen, Germany); pargyline HCl (Deutsche Abbott, Ingelheim, Germany).

RESULTS

Superfusion experiments: In superfusion experiments with neocortex slices performed at 17, 37 and 42°C, the overflow of tritium due to stimulation with 4 pulses/100 Hz (pseudo-one-pulse conditions, 76 mA, see Methods) averaged to S_1 = 0.37% of tissue tritium, [0.34 and 0.40%] (n = 47), 0.68%, [0.59 and 0.77%] (n = 65) and 0.70%, [0.65 and 0.75%] (n = 54), respectively. In hippocampal slices, which were superfused in the presence of the uptake blocker (+)-oxaprotiline (1 μM), the corresponding S_1 values (32 mA) were 0.99% of tissue tritium, [0.89 and 1.08%] (n = 60), at 22°C and 1.37% of tissue tritium, [1.27 and 1.48%] (n = 48), at 37°C. Neocortex slices superfused in the presence of the uptake blocker desipramine yielded S_1 values (with 76 mA) of 1.16% of tissue tritium, [1.04 and 1.27%] (n = 47), at 17°C and 1.49% of tissue tritium, [1.28 and 1.70%] (n = 48), at 37°C, respectively.

The S_1 values from neocortical slices treated with 360 pulses at 3 Hz amounted to 3.74% of tissue tritium, [2.99 and 4.49%] (n = 16), at 22°C and to 5.11%, [4.20 and 6.01%] (n = 22), at 37°C in the absence of (+)-oxaprotiline. The corresponding S_1 values in the presence of (+)-oxaprotiline were 4.63%, [4.12 and 5.15%] (n = 23), at 22°C and 9.02%, [7.98 and 10.06%] (n = 24), at 37°C.

When no α_2-autoreceptor agonist was used, repeated stimulation with 4 pulses/100 Hz reproducibly elicited a similar overflow of tritium at S_1, S_2, S_3 and S_4, respectively under all conditions (data not shown).

UK14304, clonidine and guanfacine, when added before S_2, S_3 and S_4, reduced the electrically evoked overflow of tritium in a concentration-dependent manner at all temperatures and stimulation parameters applied. Figure 1 shows the concentration-inhibition data of experiments with UK14304 and clonidine performed at 17, 37 and 42°C and the corresponding curve fits according to function (1). Results of the nonlinear regression

analysis of these concentration-inhibition data are summarized in Table 1 which also contains the parameter estimates from the guanfacine concentration-inhibition data (desipramine present throughout superfusion in this case; curves not shown).

It is obvious from Fig. 1 and Table 1 that the concentrations-response curves of the full agonist UK14304 were hardly affected by changing the temperature of the superfusion fluid: The parameter estimates differ neither significantly nor substantially although a tendency towards an increase in the I_{max} and pIC_{50} estimates with increasing temperature may be assumed. The partial agonists clonidine and guanfacine, however, exhibited a marked and significant increase in I_{max} from 17 to 37°C and a corresponding enhancement of the pIC_{50}s. The additional increase of the pIC_{50} of clonidine at the step from 37 to 42°C was not significant. The estimates of *c* were mostly close to unity, though, sometimes, with broad CI_{95}.

The above mentioned experiments in neocortex tissue with UK14304 and clonidine were repeated in hippocampal slices at 22 and 37°C by another experimenter, with other batches of UK14304 and clonidine, with electrical pulses of only 32 mA instead of 76 mA current strength and with superfusion fluid containing (+)-oxaprotiline. All other experimental conditions were the same (Methods). The corresponding results of Table 2 show that the parameter estimates from this approach differed from those of Table 1; the temperature-induced changes of these parameters, however, were nearly identical: very similar values in the case of the full agonist UK14304, but significant increases in I_{max} and pIC_{50} with increasing temperature in the case of the partial agonist clonidine.

α_2-Adrenoceptor ligands were also tested in neocortex slices when an autoinhibitory tone due to released NA was operative (see Methods;[7]). In this case with stimulation conditions of 360 pulses at 3 Hz endogenous NA competed with the exogenous agonists, UK14304 and clonidine, for the α_2-autoreceptors or these α_2-autoreceptors were blocked by the antagonists idazoxan or rauwolscine. The very similar increases at both temperatures, 22 and 37°C, of the evoked [^{3}H]-overflow due to the antagonists are shown in Fig. 2A. The very similar decreases at both temperatures due to UK14304 are displayed in Fig. 2B which also shows that clonidine was still inhibitory at 37°, but not at 22°C.

Saturation binding experiments: As shown in Fig. 3, specific binding of [^{3}H]-NA/NA to neocortical synaptosomes was saturable and of high affinity at both temperatures tested (22 and 37°C). Nonspecific binding, defined by using 10 μM idazoxan, increased linearly over the range of radioligand concentrations studied (not shown). Nonlinear regression analysis with function (1) was performed using all individual data points and modified by the weight of $1/(SD^2)$ at each concentration, with SD being the standard deviation of the data points at this concentration. Thus, the increase in the variation of the data with increasing ligand concentrations, which at least partly was due to the dilution of [^{3}H]-NA with unlabelled NA, was considered. The analysis revealed B_{max} values of 0.15 pmol/(mg protein), [0.12, 0.19], at 22°C and 0.18 pmol/(mg protein), [0.16, 0.22], at 37°C. The corresponding pK_d values were 8.09, [7.96, 8.22], at 22°C and 7.80, [7.70, 7.89], at 37°C. Note that the pK_d estimate at 22°C was rather higher, not lower, than that at 37°C. As the slope factors *c* (i.e. Hill coefficients) were near unity (0.88, [0.79, 0.98], respectively 1.02, [0.95, 1.10]), a single binding site at both temperatures tested could be assumed.

Competition binding experiments: UK14304, clonidine and guanfacine reduced the specific binding of [^{3}H]-NA to synaptosomal membranes in a concentration-dependent manner at 17 and 37°C in the neocortex and at

Table 1: Parameter estimates with CI_{95}s of functional data in the neocortex. Slices preloaded with [^{3}H]-NA were superfused at 17°C, 37°C and at 42°C. Four periods of electrical stimulation (S_{1-4}) elicited [^{3}H]-NA release free of autoinhibition. The autoreceptor agonists UK14304, clonidine and guanfacine were added at increasing concentrations 15 min before S_2, S_3 and S_4 to obtain concentration-inhibition data. The parameters of the corresponding curves were evaluated by nonlinear regression analysis

UK14304	17°C	37°C	42°C
I_{max} (%)	80.6 [74.8, 87.4]	85.1 [79.8, 90.9]	86.2 [76.5, 97.3]
pIC_{50}	7.76 [7.59, 7.93]	7.97 [7.83, 8.08]	8.02 [7.71, 8.25]
c	0.90 [0.63, 1.35]	1.07 [0.76, 1.61]	0.84 [0.54, 1.33]
Clonidine			
I_{max} (%)	54.3 [49.7, 59.7]	74.1 [69.3, 79.6]	73.5 [67.6, 80.4]
pIC_{50}	7.61 [7.37, 7.82]	8.15 [8.01, 8.28]	8.38 [8.20, 8.58]
c	1.10 [0.73, 1.71]	1.03 [0.76, 1.43]	1.15 [0.71, 2.21]
Guanfacine			
I_{max} (%)	38.1 [32.1, 48.8]	58.3 [52.2, 63.8]	
pIC_{50}	6.63 [5.91, 7.11]	8.18 [7.86, 8.49]	
c	0.59 [0.38, 0.98]	0.67 [0.49, 1.07]	

Table 2: Parameter estimates with CI_{95}'s of functional data in the hippocampus. Slices preloaded with [^{3}H]-NA were superfused at 25 and 37°C. Four periods of electrical stimulation (S_{1-4}) elicited [^{3}H]-NA release free of autoinhibition. The autoreceptor agonists clonidine and UK14304 were added at increasing concentrations 15 min before S_2, S_3 and S_4 to obtain concentration-inhibition data. The parameters of the corresponding curves were evaluated by nonlinear regression analysis

UK14304	25°C	37°C
I_{max} (%)	90.4 [87.6, 93.5]	90.1 [86.7, 93.9]
pIC_{50}	8.36 [8.30, 8.42]	8.27 [8.19, 8.33]
c	1.15 [0.99, 1.36]	1.13 [0.96, 1.34]
Clonidine		
I_{max} (%)	78.1 [74.1, 81.4]	84.9 [81.6, 88.6]
pIC_{50}	8.09 [8.01, 8.17]	8.42 [8.35, 8.50]
c	0.98 [0.83, 1.17]	1.01 [0.85, 1.22]

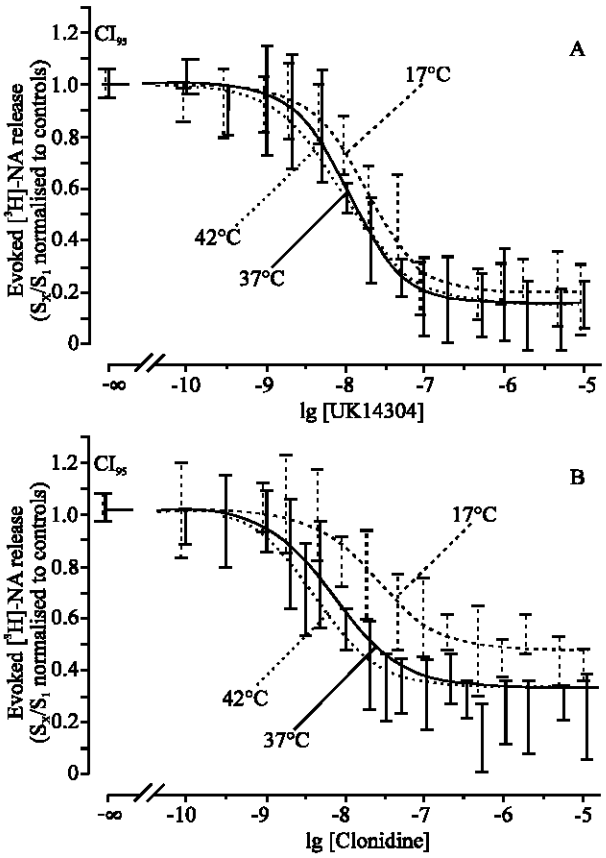


Fig. 1: Concentration-response curves of α_2-adrenoceptor agonists at different temperatures. Neocortical slices of the rat were incubated with [^{3}H]-NA at 37°C and then superfused and electically stimulated at the temperatures indicated. The inhibition of evoked [^{3}H]-NA release was normalized to corresponding controls where release was evoked in the absence of α_2-adrenoceptor agonists. CI_{95}s of the means of 5 - 14 data points per concentration are shown. For the sake of clarity, the CI_{95}s of the data at 42°C were omitted. The individual data points were subjected to nonlinear regression analysis

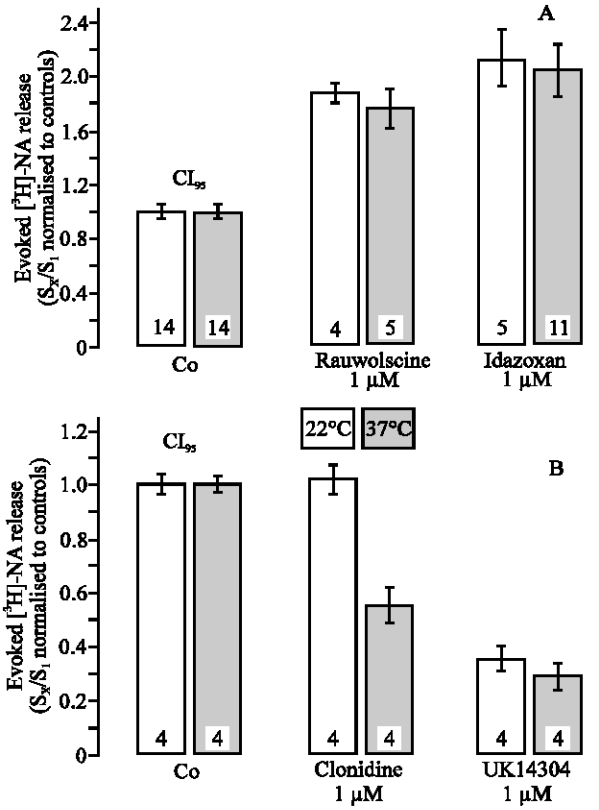


Fig. 2: Effects of α_2-adrenoceptor antagonists (rauwolscine, idazoxan, A) and agonists (clonidine, idazoxan, B) on [^{3}H]-NA release evoked with 360 electrical pulses at 3 Hz. Neocortical slices of the rat were incubated with [^{3}H]-NA at 37°C and then superfused and electrically stimulated at the temperatures indicated. The change in evoked [^{3}H]-NA release was normalized to corresponding controls where release was evoked in the absence of α_2-adrenoceptor ligands. The numbers of single observations are given in the columns

22 and 37°C in the hippocampus. Whereas superfusion experiments at 42°C were reliably possible (see above), competition binding experiments at this temperature were hampered by a large increase in nonspecific binding (see Methods). Thus, binding experiments were not performed at 42°C. Figure 4 shows the concentration-inhibition data of UK14304 and clonidine with neocortical synaptosomes performed at 17 to 37°C and the corresponding curve fits according to function (2a). Since all estimates of the slope parameter *c* in the binding experiments were near unity with CI_{95}s overlapping unity (not shown), as to be expected from bimolecular binding reactions, the parameters pK_i and I_{max} were re-estimated with function (3). This function (3) needed an estimate of pK_d of [^{3}H]-NA/NA from the saturation experiments (above). Since the two estimates of pK_d of NA at the different temperatures were very similar we introduced the mean of 8.09 and 7.80, i.e. 7.94, in addition to the used concentration of the radioligand [^{3}H]-NA ($10^{-8.30}$ M) in function (3). The results of these nonlinear regression analyses are summarized in Table 3 and 4. Table 4 also contains the corresponding estimates for guanfacine.

Obviously, in neocortex synaptosomes the increase from 17 to 37°C did not affect the binding parameters of UK14304, but markedly increased the pK_i estimates of

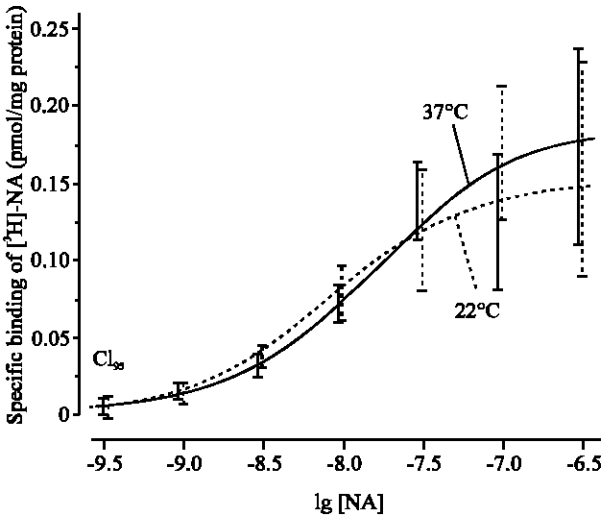


Fig. 3: Saturation characteristics of [^{3}H]-NA/NA binding to neocortical synaptosomes. Specific activity of [^{3}H]-NA was diluted with unlabelled NA to obtain final assay concentrations from 0.32 to 320 nM. Synaptosomes (~ 250 μg protein) were incubated for 60 min at 22°C or at 37°C. Non-specific binding was determined by using 10 μM idazoxan. CI_{95}s of the means of 4 - 12 data points per concentration are shown. The individual data points were subjected to nonlinear regression analysis

Table 3: Parameter estimates with CI_{95}s of binding data in the neocortex. Synaptosomes prepared from thawed brain samples were incubated with [^{3}H]-NA and various concentations of the competing drug at 17 and 37°C to obtain concentration-inhibition data. The parameters of the corresponding curves were evaluated by nonlinear regression analysis. Specific binding of [^{3}H]-NA was defined as total binding minus binding in the presence of 10 μM idazoxan

UK14304	17°C	37°C
I_{max} (%)	91.1 [86.3, 96.0]	96.3 [89.4, 103.5]
pK_i	8.51 [8.39, 8.64]	8.33 [8.16, 8.49]
Clonidine		
I_{max} (%)	92.8 [86.7, 99.0]	90.6 [85.0, 96.5]
pK_i	8.35 [8.20, 8.50]	8.85 [8.69, 9.02]
Guanfacine		
I_{max} (%)	98.8 [95.7, 102.2]	96.6 [91.0, 100.5]
pK_i	8.44 [8.34, 8.53]	8.78 [8.64, 8.91]

Table 4: Parameter estimates with CI_{95}s of binding data in the hippocampus. Synaptosomes prepared from thawed brain samples were incubated with [^{3}H]-NA and various concentations of the competing drug at 22 and 37°C to obtain concentration-inhibition data. The parameters of the corresponding curves were evaluated by nonlinear regression analysis. Specific binding of [^{3}H]-NA was defined as total binding minus binding in the presence of 10 μM idazoxan

UK14304	22°C	37°C
I_{max} (%)	91.5 [85.1, 97.1]	82.5 [74.1, 89.1]
pK_i	8.82 [8.66, 8.98]	8.50 [8.29, 8.70]
Clonidine		
I_{max} (%)	84.0 [77.1, 92.2]	87.9 [81.7, 93.4]
pK_i	8.16 [7.98, 8.35]	8.55 [8.37, 8.73]

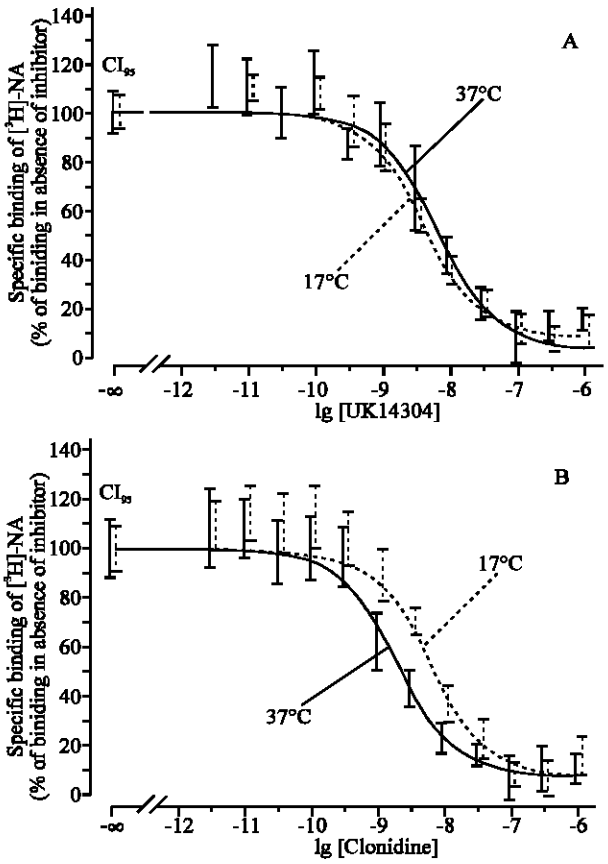


Fig. 4: Concentration-inhibition curves of α_2-adrenoceptor agonists at different temperatures. Neocortical synaptosomes of the rat were incubated at the temperatures indicated with [^{3}H]-NA in the absence or presence of α_2-adrenoceptor agonists competing for specific [^{3}H]-NA binding. The inhibition of [^{3}H]-NA binding was normalized to corresponding controls where specific binding was obtained in the absence of α_2-adrenoceptor agonists

clonidine and guanfacine. The increases were in the same order of magnitude as seen in the corresponding functional experiments. The maximum inhibition of binding was not changed by the increase in temperature for all agonists tested.

The same constellation was seen in the hippocampus: No temperature-induced increase of the pK_i of UK14304, but a large increase of the pK_i of clonidine at 37°C compared to 22°C. Again, the increases in temperature did not change the I_{max} estimates.

DISCUSSION

This study intends to explain partial agonism mechanistically, i.e. by revealing thermodynamically the different molecular interactions of a full agonist and a partial agonist with its receptor. The experimental basis of the proposed explanation is the presynaptic α_2-autoreceptor, regulating the release of NA by inhibitory feedback. Experimentally, electrically evoked [^{3}H]-overflow from rat brain slices preincubated with [^{3}H]-NA was evaluated, among others. This tritium overflow may be assumed to represent the release of the transmitter NA from noradrenergic nerve terminals (called [^{3}H]-NA release in the following). Autoinhibition by released NA was either prevented or allowed in the present experiments: Using the so-called pseudo-one-pulse conditions of electrical stimulation[7,8] estimates of I_{max} and IC_{50} of the applied exogenous α_2-adrenoceptor agonists were received, unaltered by an endogenous NA tone. Thus, with slope factors *c* near unity IC_{50} estimates could be assumed to reflect K_d values[2]. When autoinhibition was operative due to stimulation with 360 pulses at 3 Hz, the exogenous α_2-adrenoceptor ligands had to compete with endogenous NA for the α_2-autoreceptors. Thus, their agonist or antagonist property was revealed by their degree of inhibition or disinhibition, respectively, of [^{3}H]-NA release, which was already pre-inhibited by endogenous NA.

UK14304, clonidine and guanfacine were compared as agonists at α_2-autoreceptors in slices of rat neocortex and hippocampus (UK14304 and clonidine only) at different temperatures (neocortex: 17, 37 and 42°C; hippocampus: 22 and 37°C). In addition, the binding of [^{3}H]-NA, of UK14304, clonidine and guanfacine to α_2-adrenoceptor sites was also tested at different temperatures in synaptosomes prepared from the same rat brain regions. Synaptosomes, which represent nerve endings, were used instead of tissue membranes from whole brain homogenates in order to analyse as closely as possible presynaptic α_2-autoreceptor sites, corresponding to those in functional release experiments. Of course, the binding experiments could not be restricted to α_2-adrenoceptor sites representing α_2-autoreceptors on noradrenergic nerve terminals only, but may also refer to α_2-heteroreceptor sites and, partly, to postsynaptic α_2-adrenoceptor sites. However, we did not expect adulterant effects due to the inevitable heterogeneity of α_2-adrenoceptor sites analysed since pre- and postsynaptic α_2-adrenoceptors are very similar within the same species[12].

At low temperatures (17 or 22°C), the pK_ds of clonidine and guanfacine were lower, both functionally and in competition binding studies and the maximum inhibitory effects of these partial agonists on [^{3}H]-NA release were reduced. This was neither the case for UK14304 nor for [^{3}H]-NA/NA, the latter being tested in saturation binding experiments only.

The interactions with respect to temperature-induced changes between exogenous α_2-adrenoceptor ligands and endogenous NA in release experiments allowing autoinhibition were also dependent on the partial agonist property of a ligand. Clonidine, but not the full agonist UK14304, completely lost its inhibitory effect when tested at 22°C, although the degree of autoinhibition, i.e. the biophase concentration of NA, was similar at 22°C and 37°C. This was obvious from the same degree of disinhibition of [^{3}H]-NA release by the pure α_2-adrenoceptor antagonists rauwolscine and idazoxan. Note that idazoxan is a partial antagonist in the rabbit brain, but not in the rat[13,14]. Thus, only the partial agonist clonidine changed the degree of its effect with changing temperature, but neither of the pure adrenoceptor ligands, the agonists UK14304 and the transmitter NA and the antagonists rauwolscine and idazoxan.

The results on the temperature-dependent effects of clonidine under autoinhibition clearly disprove an earlier explanation which emphasized the pivotal role of the biophase concentration of the released transmitter for the action of a partial agonist at the presynaptic receptor[15]. This statement was exactly the reason why we especially analysed clonidine and the other partial agonist, guanfacine, under autoinhibition-free conditions. Thus, we intend to explain the action of a partial agonist at the α_2-autoreceptor without any interaction with the released transmitter: The identical disinhibitory actions at both 22 and 37°C of the full antagonists rauwolscine and idazoxan indicate a very similar biophase concentration at 22 and 37°C, which therefore cannot explain the different behaviour of clonidine at these temperatures. The same results from the similar inhibition of the full agonist UK14304 in comparison to the temperature-dependent action of the partial agonist clonidine.

What is the reason for this unique behaviour of a partial agonist, which - as we speculatively believe - is not confined to clondine and guanfacine, but may be assumed for all partial (ant)agonists?

We are aware that the whole chain of events, from binding of the agonist to the inhibition of the electrically evoked [^{3}H]-NA release, was subjected to the changes in temperature. The absolute values of the binding K_ds and the release IC_{50}s were dissimilar, although the latter may be assumed to reflect K_ds (since $c \approx 1$, see Methods;[2]), probably because the used buffers and other assay conditions were not the same. However, the temperature-

induced changes of the K_ds of clonidine and guanfacine can sufficiently and completely be explained at the binding level since the intervals between the K_ds of these partial agonists at the different temperatures in binding studies were rather similar to the intervals between their IC_{50}s at the different temperatures in release studies. The autoreceptor modulation of NA release was fully operative at all temperatures applied, as concluded from the similar maximum inhibition of the full agonist UK14304 at all temperatures and from the similar disinhibition of release by the antagonists rauwolscine and idazoxan at 22 and 37°C, reflecting the inhibitory effect of the full endogenous agonist NA. Whatever the influences of the different temperatures on the processes within the black box between agonist binding and response may be, the parameter estimates of *c* near unity in functional experiments and the parallelism between the pIC_{50} changes in the functional - and the pK_i changes in the competition binding - experiments suggest the following: (I) Direct proportionality between binding and response was preserved at all temperatures. (II) The obvious effects of changing the temperature happened at the binding level, i.e. at the level of the association to and dissociation from the α_2-adrenoceptors of UK14304, clonidine and guanfacine and did not differently affect downstream events of the release regulation by the full or the partial agonist.

Although the S_1 values of the release experiments were higher at 37 and 42°C than at 17 or 22°C and also reflected the absence or presence of NA uptake blockers, the statement, that downstream events of the release regulation do not explain the different behaviour of UK14304 and endogenous NA on the one side and of clonidine and guanfacine on the other, seems true for two reasons: Release of [^{3}H]-NA either occurred without autoinhibition, i.e. both the higher release at higher temperature and the lower release at lower temperature did not activate the α_2-autoreceptor, irrespective of the absence or presence of NA uptake blockers, and the degree of autoinhibition with 360 pulses at 3 Hz was the same at low and high temperature. The latter was revealed by the highly similar disinhibitory effects of the antagonists at 22 and 37°C.

With respect to direct proportionality between agonist binding and response and the equalisation $IC_{50} = K_d$[2], we may consider the equilibrium constant K_d kinetically: The binding of the agonists tested to α_2-adrenoceptors is bimolecular in nature according to the scheme:

$$[\text{Agonist}]+[\text{Receptor}] \rightleftarrows [\text{Agonist Receptor}] \quad \text{with } K_d = \frac{k_{-1}}{k_{+1}}.$$

A decrease in temperature, e.g. from 37 to 17°C, more markedly decreases the association process than the dissociation process: According to the Brownian motion, a collision, as prerequisite of binding, between agonist and receptor becomes much less likely due to the lower kinetic energies of two molecules (though one of them, the receptor, is integrated in a biological membrane) than the dissociation of the complex [Agonist Receptor] as a temporary single molecule, embedded in the cellular membrane. The temperature-dependence of the dissociation of this complex (first order reaction) is certainly less pronounced than that of the collision of two molecules (reaction of higher order). The influence of a reduction in temperature on the mobilities of the unbound imidazoline molecules, UK14304 (MW 292.1) and clonidine (MW 230.1), is most likely analogical due to their similar structure and molecular weight. This may also be true for the third agonist tested, guanfacine (MW 246.6) which structurally resembles the imidazolines. Thus, the association velocity of all three agonists with the receptor may be similar since these reactions mainly depend on the mobility of the combining molecules, agonist and receptor. The dissociations of the complexes [UK14304 Receptor], [Clonidine Receptor] and [Guanfacine Receptor], however, are not due to collisions since the mobility of receptors within the plasma membrane is limited, but depend on the stability and the kinetics of the complexes at the different temperatures.

Therefore, we assumed semi-quantitatively that the decrease in temperature from 37 to 17°C largely decreases $k_{+1(37)}$ to $k_{+1(17)}$, similarly for all three agonists. When we concentrate on clonidine and UK14304, $k_{+1(37)Cl}$ changes to $k_{+1(17)Cl}$ and $k_{+1(37)UK}$ changes to $k_{+1(17)UK}$:

$$\frac{k_{+1(37)UK}}{k_{+1(17)UK}} = \frac{k_{+1(37)Cl}}{k_{+1(17)Cl}} \gg 1$$

Thus, in order to explain the increase in the clonidine equilibrium constant K_d we have to focus on the dissociation reaction which seems different for UK14304 (and NA) on the one side and for clonidine (and guanfacine) on the other. How can we explain that?

Experimentally we found the IC_{50}s, reflecting K_ds and the K_is of clonidine and guanfacine increased when the temperature was lowered: In the neocortex, the functional IC_{50} estimates at 37°C were significantly enhanced at 17°C (Table 1) and the corresponding K_is of Table 3 also changed with temperature lowered from 37 to 17°C. The same was true for hippocampus tissue (Table 2 and 4). In contrast, the IC_{50}s and K_is of UK14304 did not show these parallel changes but rather remained constant. Let us oppose only clonidine and UK14304 in the following:

$$K_{d(17)UK} = \frac{k_{-1(17)UK}}{k_{+1(17)UK}} = \frac{k_{-1(37)UK}}{k_{+1(37)UK}} = K_{d(37)UK}$$

and

$$K_{d(17)CI} = \frac{k_{-1(17)CI}}{k_{+1(17)CI}} > \frac{k_{-1(37)CI}}{k_{+1(37)CI}} = K_{d(37)CI}$$

which can be written as:

$$\frac{k_{+1(37)UK}}{k_{+1(17)UK}} > \frac{k_{-1(37)UK}}{k_{-1(17)UK}} \quad (4)$$

and

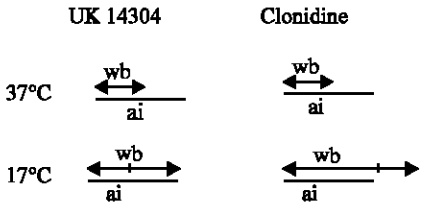
$$\frac{k_{+1(37)CI}}{k_{+1(17)CI}} > \frac{k_{-1(37)CI}}{k_{-1(17)CI}} \quad (5)$$

Thus, we have to state that the dissociation rate constant of UK14304 changes, i.e. most probably, decreases with lower temperature to the same degree as its association rate constant. This leaves the K_d of UK14304 independent of temperature changes. However, $k_{-1(37)Cl}$ less markedly decreases to $k_{-1(17)Cl}$ in comparison to the relative decrease of $k_{-1(37)UK}$ to $k_{-1(17)UK}$. In other terms, $k_{-1(17)Cl} < k_{-1(37)Cl}$ and $k_{-1(17)UK} << k_{-1(37)UK}$ This conclusion is based on experimental results and would also be true for the comparison of the full agonist UK14304 (or NA) with the partial agonist guanfacine.

The rate constant k_{-1} represents the velocity of the dissociation reaction; therefore it can be used to obtain information on the mean duration of the binding of the agonists to the α_2-autoreceptors. The whole binding duration of an agonist (wb) is indirectly proportional to its k_{-1} value, $wb \sim 1/k_{-1}$. Thus, with

$$1 << \frac{k_{-1(37)UK}}{k_{-1(7)UK}} > \frac{k_{-1(37)CI}}{k_{-1(17)CI}} > 1, \text{see}(4), (5)$$

$k_{-1(17)Cl} < k_{-1(37)Cl}$, but $k_{-1(17)UK} << k_{-1(37)UK}$, correspondingly $wb_{(17)Cl} > wb_{(37)Cl}$ and $wb_{(17)UK} >> wb_{(37)UK}$. The whole binding duration of an agonist should be somehow linked to its full or partial antagonist property as we know that a pure antagonist is characterized by pure binding only, without any activation. It is clear that for each agonist that binding is a prerequisite of receptor activation. The full agonist - partial (ant)agonist - full antagonist continuum has been defined earlier as conditional probability to activate a receptor given the ligand occupies the receptor, p(activation binding), with p = 1 in the case of a full agonist and p = 0 in the case of a full antagonist[7]. Since a partial agonist, like clonidine or guanfacine, represents both aspects of a ligand, the markedness of which depends on the temperature according to the present findings, temperature must influence properties of a partial agonist, which are responsible for the degree of partial agonism. These considerations and the train of thoughts from the experimentally founded changes of dissociation constants to the whole binding duration of ligands suggest that we have to differentiate between the mentioned whole binding duration wb and an activation interval ai. ai is the phase during which an occupied receptor can be activated and may mainly be determined by the receptor, not by a docking agonist, since it is the receptor which mediates the effect, not the triggering agonist. Therefore, ai is assumed to be for all agonists and ai similar ai decreases with increasing temperature, $ai_{(17)} > ai_{(37)}$. The following cartoon considers the relations deduced above, i.e. $wb_{(17)Cl} > wb_{(37)Cl}$ and $wb_{(17)UK} >> wb_{(37)UK}$ with $ai_{(17)}$ markedly overlapping $wb_{(37)UK}$:



Since clonidine is already a partial agonist at 37°C, $wb_{(37)Cl}$ has to be slightly longer than $ai_{(37)}$, $wb_{(37)Cl} > ai_{(37)}$. Correspondingly, $wb_{(17)Cl} > wb_{(37)Cl}$ leads to $wb_{(17)Cl} > ai_{(17)}$ (according to the semi-quantitative consideration: $wb_{(17)Cl} > wb_{(37)Cl} > ai_{(37)} < ai_{(17)}$, or ">+>+<" ≈ ">"). The same can be deduced for the partial agonist guanfacine. UK14304, however, is still a full agonist at 17°C ($ai_{(17)} \geq wb_{(17)UK}$). As $wb_{(17)UK} >> wb_{(37)UK}$, $ai_{(17)} \geq ai_{(37)}$ and $ai_{(17)} \geq wb_{(17)UK}$, $ai_{(37)} >> wb_{(37)UK}$ (consider $ai_{(37)} < ai_{(17)} \geq wb_{(17)UK} >> wb_{(37)UK}$ or "< + ≥ + >>" ≈ ">>"). The different distances wb and ai in the cartoon above in the end corresponded to our experimental findings.

The positive difference between wb and ai is the pure binding (= antagonist, competition) interval. Clonidine as partial agonist thus has a positive pure binding interval (wb_{C1}-ai>0) both at 37°C and at 17°C. UK14304, however, hardly has a positive pure binding interval at any temperature, since - as a full agonist - it never binds to the receptor without concomitant activation (wb_{UK} - *ai* <0). The decrease in the maximum effect of clonidine at 17°C is due to an increase in the ratio $wb_{(17)Cl}/ai_{(17)}$ as compared to $wb_{(37)Cl}/ai_{(37)}$ which corresponds to $[wb_{(17)Cl}-ai_{(17)}] > [wb_{(37)Cl}-ai_{(37)}]$. This means that clonidine acts as an agonist initially after docking, whereas afterwards it is still bound, preventing the access of other free clondine molecules, but, remaining in the bound state, cannot activate the receptor a second or third time.

Generally, the condition $[wb_{(17)} - ai_{(17)}] > [wb_{(37)} - ai_{(37)}]$ of a partial agonist enhances its antagonist property at low temparature since the docking of another, free agonist molecule (with ensuing receptor activation during its ai) is competitively inhibited ($wb_{(17)partial\ agonist} >> ai_{(17)}$ if $wb_{(37)partial\ agonist} > ai_{(37)}$).

The lower inhibitory effects of clonidine at submaximum concentrations can additionally be explained by $[Cl\ R]_{17} < [Cl\ R]_{37}$.

The concentration of the complex [Clonidine Receptor] is lower at 17°C than at 37°C, i.e. $[Cl\ R]_{17} < [Cl\ R]_{37}$ since

$$\frac{k_{-1(37)Cl}}{k_{+1(37)Cl}} = \frac{[Cl]\cdot[R]_{37}}{[Cl\ R]_{37}} < \frac{k_{-1(17)Cl}}{k_{+1(17)Cl}} = \frac{[Cl]\cdot[R]_{17}}{[Cl\ R]_{17}}$$

with [Cl] being the concentration of added clonidine which is predetermined and, therefore, equal in a superfusion system at both temperatures (Zone A phenomenon[16]) and [R] being the concentration of free receptors. As $[R_t]$ is the total concentration of receptors, which does not change with temperature, $[R]_{37} = [R_t] - [Cl\ R]_{37}$ and $[R]_{17} = [R_t] - [Cl\ R]_{17}$. This yields, after some few conversions, $[Cl\ R]_{17} < [Cl\ R]_{37}$. At 17°C the fraction of receptor-bound clonidine is lower than that at 37°C whereas the corresponding fractions of UK14304 are the same. The consequence of $[Cl\ R]_{17} < [Cl\ R]_{37}$ becomes obvious in the effects of clonidine at submaximum concentrations (see below), a condition similarly true for guanfacine.

Thus, at 17°C a lower fraction of receptor-bound, submaximally concentrated clonidine, $[Cl\ R]_{17}/[R_t]$, represents the condition of $wb_{(17)Cl} >> ai_{(17)}$ as compared to the higher fraction $[Cl\ R]_{37}/[R_t]$ with $wb_{(37)Cl} > ai_{(37)}$. This is in line with the experimental fact that the distance in direction of the response axis (i.e. the vertical y-axis, Fig. 1B) between the concentration-inhibition curves of clonidine at 17°C and at 37°C is larger in the steep parts of the curves than at the maximum concentrations of clonidine. In the steep parts of the curves two factors contribute to the alleviation of the inhibitory effect of clonidine at 17°C: (1) $wb_{(17)Cl}/ai_{(17)} > wb_{(37)Cl}/ai_{(37)}$ and (2) $[Cl\ R]_{17} < [Cl\ R]_{37}$, whereas at maximum clonidine concentrations nearly all receptors are occupied and only $wb_{(17)Cl}/ai_{(17)} > wb_{(37)Cl}/ai_{(37)}$ explains the alleviated I_{max}.

As mentioned above already, the concept of ai<wb explaining the phenomenon of partial agonism is in line with the view of partial agonism as conditional probability[1,2]. With ai and wb this conditional probability p(activation binding) can be expressed as p = ai/wb with ai < wb in the case of a partial agonist. Since, in a spare receptor-free system, p can also be expressed as ratio of the maximum effect of the concentration-response curve of a partial agonist (I_{max-p}) divided through the maximum effect obtained by a full agonist (I_{max-f})[2], ai/wb can be quantified as I_{max-p}/I_{max-f}. In our examples, this means $ai_{(17)}/wb_{(17)Cl} = 54.3/80.6\%$ and $ai_{(17)}/wb_{(17)Guanf} = 38.1/80.6\%$ (Table 1); in other words, $wb_{(17)Cl}$ exceeds $ai_{(17)}$ by about 33% and $wb_{(17)Guanf}$ exceeds $ai_{(17)}$ by about 53%. At 37°C, $ai_{(37)}/wb_{(37)Cl} = 74.1/85.1\%$ and $ai_{(37)}/wb_{(37)Guanf} = 58.3/85.1\%$; thus at this temperature wb_{Cl} exceeds ai by about 13% and wb_{Guanf} exceeds ai by about 31% only.

Of course, other assumptions on the activation interval are conceivable, e.g. multiple activation steps during the activation interval. However, these do not restrict our proposed concept. Interestingly, this concept is also supported by some findings of others who did not, however, interpret their data in the way of the present manuscript:

Miller *et al.*[17] investigated the binding to nicotinic acetlycholine receptors (nAChR) of the partial agonist choline at different temperatures: They were able to measure/calculate the dissociation constant K_d, the lifetimes of the complex [choline-nAChR] and the dissociation rate constants k_{-1}:

	K_d	k_{-1}	Lifetime of complex	k_{+1}
18°C	300 μM	$1.6 \cdot 10^3\ s^{-1}$	$0.625 \cdot 10^{-3}$ s	$5.33 \cdot 10^{-6}\ s^{-1}\ M^{-1}$
33.5°C	100 μM	$4.35 \cdot 10^3\ s^{-1}$	$0.23 \cdot 10^{-3}$ s	$43.5 \cdot 10^{6}\ s^{-1}\ M^{-1}$

In line with the proposals of the present manuscript, the lifetime of [choline-nAChR] decreased with increasing temperature, as displayed in the cartoon above for the case of clonidine. In addition, the decrease in temperature from 33.5 to 18°C largely decreased $k_{+1(33.5)}$ to $k_{+1(18)}$ with the consequence that:

$$\frac{k_{+1(33.5)Choline}}{k_{+1(18)Choline}} = 8.16 \gg 1$$

as deduced above ($k_{+1(37)Cl} >> k_{+1(17)Cl}$).

In summary, a rather obvious and simple conclusion can be drawn from the present results, which showed that a decrease in temperature enhanced the antagonist property of a partial agonist, leaving the full agonist property unaffected: The whole binding duration may be larger than the activation interval of the receptor in the case of a partial agonist, but lower in the case of a full agonist. Binding studies confirmed that the effects of changing the temperature can be fully explained on the level of the ligand-receptor interaction. The temperature-dependent relation between whole binding duration and activation interval of a partial agonist is in

line with the assumption that the dissociation of a partial aginist-autoreceptor complex is less probable than that of a full agonist-autoreceptor complex at all temperatures.

This summary does not confine itself to conditions of α_2-adrenoceptors although our functional approach only refers to presynaptic α_2-autoreceptors. We think, however, that the underlying thermodynamic principles could generally explain the phenomenon of partial agonism at receptors of the plasma membrane. This assumption, however, still needs experimental confirmation.

ACKNOWLEDGEMENT

We thank Pfizer Inc.,USA, for providing financial support.

REFERENCES

1. Feuerstein, T.J., W. Sauermann, C. Allgaier, E. Agneter and E.A. Singer, 1994. New insights into receptor theory, as provided by an artificial partial agonist made-to-measure. Naunyn-Schmiedeberg´s Arch. Pharmacol., 350: 1-9.
2. Feuerstein, T.J. and N. Limberger, 1999. Mathematical analysis of the control of neurotransmitter release by presynaptic receptors as a supplement to experimental data. Naunyn-Schmiedeberg´s Arch. Pharmacol., 359: 349-359.
3. Colquhoun, D., 1998. Binding, gating, affinity and efficacy: The interpretation of structure-activity relationships for agonists and of the effects of mutating receptors. Br. J. Pharmacol., 125: 924-947.
4. Del Castillo, J. and B. Katz, 1957. Interaction at end-plate receptors between different choline derivatives. Proc. Roy. Soc. Lond. B., 146: 369-381.
5. Schwartz, T.W. and A.P. Ijzerman, 1998. Principles of agonism: Undressing efficacy. Trends Pharmacol. Sci., 19: 433-436.
6. MacLennan, S.J., L.A. Luong, J.R. Jasper, Z.P. To and R.M. Eglen, 1997, Characterization of α_2-adrenoceptors mediating contraction of dog saphenous vein: Identity with the human α_{2A} subtype. Br. J. Pharmacol., 121: 1721-1729.
7. Allgaier, C., E. Agneter, T.J. Feuerstein and E.A. Singer, 1992. Estimation of the biophase concentration of noradrenaline at presynaptic α_2-adrenoceptors in hippocampus and cortex. Naunyn-Schmiedeberg´s Arch. Pharmacol., 345: 402-409.
8. Singer, E.A., 1988. Transmitter release from brain slices elicited by single pulses: A powerful method to study presynaptic mechanisms. Trends Pharmacol. Sci., 9: 274-276.
9. U´Prichard, D.C. and S.H. Snyder, 1978. ^{3}H-Catecholamine binding to α-receptors in rat bran: enhancement by reserpine. Eur. J. Pharmacol., 51: 145-155.
10. Cheng, Y.C. and W.H. Prusoff, 1973. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (I_{50}) of an enzymatic reaction. Biochem. Pharmacol., 22: 3099-3108.
11. Altman, D.G., 1991. Statistics in medical journals: developments in the 1980s. Stat. Med., 10: 1897-1913.
12. Starke, K., 2001. Presynaptic autoreceptors in the third decade: focus on α_2-adrenoceptors. J. Neurochem., 78: 685-693.
13. Heepe, P. and K. Starke, 1985. α-adrenoceptor antagonists and the release of noradrenaline in rabbit cerebral cortex slices: Support for the α-autoreceptor hypothesis. Br. J. Pharmacol., 84: 147-155.
14. Limberger, N. and K. Starke, 1983. Partial agonist effect of 2-[2-(1,4-benzodioxanyl)]-2-imidazoline (RX 781 094) at presynaptic α_2-adrenoceptors in rabbit ear artery. Naunyn-Schmiedeberg´s Arch. Pharmacol., 324: 75-78.
15. Cichini, G., H. Lassmann, P. Placheta and E.A. Singer, 1986. Effects of clonidine on the stimulation-evoked release of ^{3}H-noradrenaline from superfused rat brain slices as a function of the biophase concentration. Temperature dependent widening of extracellular space. Naunyn-Schmiedeberg´s Arch. Pharmacol., 333: 36-42.
16. Straus, O.H. and A. Goldstein, 1943. Zone behaviour of enzymes. J. Gen. Physiol., 26: 559-579.
17. Miller, J., V. Witzemann, U. Quast and M.A. Raftery, 1979. Proton magnetic resonance studies of cholinergic ligand binding to the acetylcholine receptor in its membrane environment. Proc. Natl. Acad. Sci. USA., 76: 3580-3584.