Modification of Noradrenaline Release in Pithed Spontaneously Hypertensive Rats by $I_1$-Binding Sites in Addition to $\alpha_2$-Adrenoceptors

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ABSTRACT

It is known that moxonidine acts as an agonist at presynaptic $\alpha_2$-adrenoceptors of the postganglionic sympathetic nerve terminals and leads to a reduction in noradrenaline release. In addition, it is conceivable that $I_1$-binding sites located in other regions of the pre- and postganglionic sympathetic neurons are involved in this effect. Our aim was to investigate whether and to what extent activation of the $I_1$-binding sites contributes to inhibition of noradrenaline release. Noradrenaline release was induced in pithed spontaneously hypertensive rats (pretreated with phenoxybenzamine/desipramine at 10/0.5 mg/kg) by stimulation of sympathetic overflow from the spinal cord. Noradrenaline overflow was reduced using moxonidine (0.18, 0.6, and 1.8 mg/kg) by 39.4, 70.4, or 78.7%, respectively, even when all $\alpha_1$/$\alpha_2$-adrenoceptors were blocked effectively by phenoxybenzamine. In contrast, the $I_1$-antagonist efaroxan (0.1, 1, and 3 mg/kg) increased noradrenaline overflow from 453 (control) to 1710, 1999, or 2754 pg/ml, suggesting an autoreceptor-like function of $I_1$-binding sites. In consequence, moxonidine (0.18, 0.6, and 1.8 mg/kg) reduced the increase in noradrenaline overflow in efaroxan-treated animals (1 mg/kg) by 22.7, 41.7, and 50.5%, respectively. Agmatine (6 and 60 mg/kg), an endogenous agonist at $I_1$-binding sites, reduced noradrenaline overflow (78.7%, respectively, even under $\alpha_2$-adrenoceptor blockade. When 2-endo-3-exo-isopropylbicyclo[2.2.1]heptane (AGN192403) (10 mg/kg) was injected, a selective blocker of $I_1$-binding sites, noradrenaline overflow was not influenced by agmatine. It is concluded that moxonidine reduces noradrenaline overflow by acting at $I_1$-binding sites in addition to its agonistic property at $\alpha_2$-adrenoceptors. The exact location of the $I_1$-binding sites on the pre- or postsynaptic sympathetic neurons is unknown, but the location in the pre- or postsynaptic membrane of the sympathetic ganglion is the most plausible explanation.

Clonidine and the related compounds moxonidine and rilmenidine that induce inhibition of noradrenaline release from sympathetic neurons are second line antihypertensives. Recently, the presynaptic $\alpha_2$-adrenoceptor mediating an inhibition of noradrenaline release from sympathetic nerve terminals was subclassified as the $\alpha_{2A}$- and $\alpha_{2C}$-adrenoceptor (Altman et al., 1999). In addition, clonidine and other imidazolines have been suggested to modulate noradrenaline release via non-$I_1$-/non-$I_2$-presynaptic imidazoline binding sites, which were identified on the sympathetic axon terminals of rabbit, rat, guinea pig, and human cardiovascular tissue (Göthert et al., 1999; Molderings and Göthert, 1999). In contrast, investigations by other authors revealed that these drugs inhibit noradrenaline release exclusively by activating prejunctional $\alpha_2$-adrenoceptors (Bohmann et al., 1994; Gaiser et al., 1999). Whether imidazoline binding sites are involved (in addition to $\alpha_2$-adrenoceptors) in noradrenaline release may be dependent on stimulation conditions (Molderings et al., 1999a) and/or species differences (Molderings et al., 2000).

Such results have been based mainly on in vitro studies appropriate for the identification of a drug’s presynaptic site of action. However, to what extent do such in vitro results apply in vivo? Studies in conscious and pithed rabbits have supported the view that central sympathetic inhibition is mediated only via $\alpha_2$-adrenoceptors (Urban et al., 1995; Bock et al., 1999; Szabo et al., 1999). In pithed, spontaneously hypertensive rats (SHR) and rabbits, rilmenidine and moxonidine decreased the stimulated overflow of noradrenaline (Häuser et al., 1995; Urban et al., 1995; Szabo et al., 1999). Because imidazoline derivatives were still able to reduce noradrenaline overflow dose dependently after rauwolscine pretreatment, an $\alpha_2$-adrenoceptor-independent mechanism was suggested (Häuser et al., 1995). However, it seems not unlikely that clonidine, moxonidine, or rilmenidine induces sympathetic inhibitory effects via $\alpha_2$-adrenoceptors even in the presence of rauwolscine, because this $\alpha_2$-blocker was

ABBREVIATIONS: SHR, spontaneously hypertensive rats; KO, knockout; PE, polyethylene; nAch, nicotinic acetylcholine.
characterized to be a competitive antagonist (Bock et al., 1999), which would strengthen the idea of an imidazoline binding site-independent regulation of noradrenaline release. The best way to show in vivo whether imidazoline binding sites would have some impact in regulating noradrenaline release is to use $\alpha_2$-adrenergic receptor knockout (KO) mice (Hein, 2001). However, the residual $\alpha_2$-mediated effect in the $\alpha_{2A/AD}$-KO mice suggests that the $\alpha_{2C}$-adrenoceptor also functions as a presynaptic autoreceptor for inhibiting noradrenaline release (Altman et al., 1999). Because only the $\alpha_{2A/C}$-double KO mouse (an animal model that was not available to us) would serve as a suitable experimental tool to answer this question, we decided to overcome the limitation of a competitive blockade of $\alpha_2$-adrenoceptors by performing experiments on reduced noradrenaline overflow evoked by clonidine-like substances (e.g., moxonidine) under irreversible $\alpha_2$-adrenoceptor blockade in pithed SHR. This rat strain is a well established model of sympathetic hyperactivity, which represents another rationale for using SHR instead of mice.

Using an irreversible blocker of $\alpha_2$-adrenoceptors (phenoxybenzamine) and a specific ligand for imidazoline binding sites (2-endo-amino-3-exo-isopropylbicyclo[2.2.1]heptane; AGN192403), the aim of this study was to determine 1) whether imidazoline binding sites contribute to the moxonidine-induced modification of noradrenaline release in vivo and, if so, 2) to specify the subtype of imidazoline binding sites involved in this effect.

Materials and Methods

Animal Preparation. The present study was conducted according to the declaration of Helsinki, following the guidelines for the care and use of laboratory animals as adopted by the Ministerium für Natur und Umwelt des Landes Schleswig Holstein, Deutschland, animal protocol no. 9/A4/81. Male, spontaneously hypertensive rats (Charles River, Sulzfeld, Germany), weighing 200 to 250 g, were pithed under ether anesthesia using a steel rod (1.5 mm diameter) coated with enamel except for the length of the thoracolumbar spinal cord (Th4-Th12 segments) as described by Gillespie and Muir (1967). A steel cannula was inserted as an indifferent electrode into the dorsal subcutis located near the lumbar vertebral column. Both vagal nerves were cut at the neck, and neuromuscular junctions were blocked by d-tubocurarine (3 mg/kg). Polyethylene catheters were inserted into both femoral veins (PE-10) for drug administration and into both carotid arteries (PE-50) for measuring blood pressure and collecting blood samples. The polyethylene catheter (PE-50), which was inserted into the left carotid artery, was connected to a Statham P23 Db pressure transducer (Hellige, Freiburg, Germany). Blood pressure and heart rate were recorded continuously and sampled digitally.

Influence of Moxonidine, Efaroxan, and Idazoxan on Blood Pressure Response. Dose-response curves for blood pressure were generated for moxonidine, efaroxan, and idazoxan over concentrations ranging between 0.1 and 10,000 $\mu$g/kg by cumulative bolus injections. The ED$_{50}$ values for moxonidine’s dose-response curves were calculated. All subsequent stimulation experiments were performed using 3, 10, or 30 times the ED$_{50}$ value of moxonidine.

Stimulation Experiments. Electrical stimulation of the thoracolumbar portion of the spinal cord was performed at 10 V (1-ms pulse duration at 0.5 Hz for 3 min), applied via the pithing rod. After preparation, pithed SHR were allowed to recover until blood pressure and heart rate were constant. Before any stimulation, all rats were pretreated with desipramine (0.5 mg/kg) to inhibit neuronal catecholamine uptake and, when appropriate, phenoxybenzamine (3 or 10 mg kg$^{-1}$) to block $\alpha_2$-adrenoceptors. Depending on the protocol, pretreatment of SHR also included a short-lasting infusion within 30 s of either efaroxan (0.1 or 1.0 mg/kg) or idazoxan (0.01 or 0.1 mg/kg). Thereafter, either moxonidine or solvent (as a control) was infused over 10 min. Seven minutes after starting the infusion, rats were stimulated for 3 min as described above. During the last 30 s of stimulation, blood (1 ml) was sampled from the carotid artery. The loss in volume was compensated for by the infusion of hydroxyethyl starch (6%; Fresenius, Homburg, Germany) (1 ml) over 5 min. Before infusing a higher dose of moxonidine, animals were allowed to recover for at least 10 min.

In another set of experiments, the influence of agmatine on noradrenaline overflow was tested. Wherever appropriate, animals were pretreated 10 min before agmatine infusion (6, 33, or 60 mg/kg) either with phenoxybenzamine (10 mg/kg; infusion within 30 s) or AGN192403 (10 mg/kg, infusion within 30 s) to block $\alpha_2$-adrenoceptors or I$_1$-binding sites, respectively. Within the last 30 s of the 2-min agmatine infusion, global sympathetic outflow (C7-L3) was induced by preganglionic electrical stimulation (20 V, 1-ms pulse duration at 0.5 Hz for 0.5 min) via the steel rod before blood (1 ml) was sampled for noradrenaline analysis.

Noradrenaline Determination. Blood samples were centrifuged at 4°C for preparing plasma. Plasma noradrenaline as an index of sympathetic overflow was measured by high-performance liquid chromatography and electrochemical detection after plasma (350 $\mu$l) was adsorbed onto alumina in a Tris-buffer system (700 $\mu$l), consisting of 1.5 M tri(hydroxy-methyl)aminomethane hydrochloride, 68 nM EDTA, and 3.6 mM glutathione) and eluted with 100 $\mu$l of 0.1 M perchloric acid using 500 pg of dihydroxybenzylamine as an internal standard.

Substances. Moxonidine was a generous gift from Solvay (Hanover, Germany). Efaroxan, idazoxan, and phenoxybenzamine were obtained from Sigma/RBI (Natick, MA); AGN192403 was from Torcix (Bristol, UK); and agmatine, d-tubocurarine, and desipramine were from Sigma (Deisenhofen, Germany). All other chemicals (HPLC or analytical grade) were purchased either from Sigma Chemie (Deisenhofen, Germany) or Merck (Darmstadt, Germany).

Statistical Analysis. Data shown in tables and figures are expressed as means ± S.E.M. ED$_{50}$ values were calculated by nonlinear ear curve fitting (GraphPad Prism; GraphPad Software, Inc., San Diego, CA). Statistical analysis was performed by one-way analysis of variance followed by appropriate post hoc tests (Bonferroni’s multiple comparison test). A significance level of 0.05 or less was considered to represent a statistically significant difference.

Results

Effects of Drugs on the Blood Pressure and Heart Rate of Pithed SHR. In the following experiments, both diastolic and systolic blood pressure were monitored via a carotid catheter. Changes induced by various drugs were similar for diastolic and systolic blood pressure. For reasons of clarity and because changes in blood pressure of pithed SHR are related more to peripheral resistance (which is reflected more by the diastolic blood pressure), only this parameter is depicted in the following figures. Dose-response curves of moxonidine, efaroxan, and idazoxan were determined for their increasing effect on diastolic blood pressure (Fig. 1A): the ED$_{50}$ values were 60 ± 15, 28 ± 9, and 16 ± 9 $\mu$g/kg, respectively. The maximal increase ($E_{\text{max}}$) of diastolic blood pressure was 136 ± 5 mm Hg using moxonidine, but considerably less in the case of efaroxan (24 ± 2 mm Hg) or idazoxan (30 ± 8 mm Hg). The heart rate decreased slightly under moxonidine infusion, whereas efaraxon and idazoxan actually increased the heart rate slightly (Fig. 1B). All subsequent stimulation experiments were performed using mox-
Means pithed spontaneously hypertensive rats. Values are expressed as zoxan (H11006) on diastolic blood pressure (DBP; A) and heart rate (B) in

Fig. 1. Dose-response curves for moxonidine (O), efaroxan (△), and idazoxan (□) on diastolic blood pressure (DBP; A) and heart rate (B) in

Effectiveness of the Phenoxybenzamine-Induced α2-Adrenoceptor Blockade. In a further set of experiments in pithed SHR, the plasma noradrenaline concentration after electrical stimulation of preganglionic sympathetic nerves (designated as noradrenaline overflow in this and subsequent sections) was determined. Plasma noradrenaline concentrations after electrical stimulation without phenoxybenzamine pretreatment were 95 ± 8.4 pg/ml (Fig. 2A). These plasma noradrenaline levels were increased 5- to 6-fold after blocking α2-adrenoceptors with phenoxybenzamine (3 or 10 mg/kg). Higher phenoxybenzamine doses did not further increase noradrenaline overflow (Fig. 2A). Evoked noradrenaline overflow remained unchanged at the four stimulation periods (Fig. 2C). The stimulation-evoked elevation of diastolic blood pressure without phenoxybenzamine pretreatment was 16.5 ± 2.7 mm Hg and tended to decrease over the

Influence of Efaroxan and Idazoxan on the Stimulated Noradrenaline Overflow. The electrically stimulated plasma noradrenaline concentration without phenoxybenzamine treatment was 87.6 ± 8.2 pg/ml (Fig. 3A), consistent with values (95.4 ± 8.8 pg/ml) obtained from experiments where the dependence of plasma noradrenaline concentration on phenoxybenzamine dose was demonstrated (Fig. 2A). This level was almost halved by moxonidine under all dose regimes. The elevation of electrically stimulated plasma noradrenaline levels under α2-adrenoceptor blockade was reduced by moxonidine dose dependently to values (96.4 pg/ml) similar to those observed in phenoxybenzamine- and moxonidine-free animals (87.6 ± 8.2 pg/ml; Fig. 3A). Complete α2-adrenoceptor blockade was confirmed by the lack of blood pressure response, because the moxonidine- and stimulation-dependent increase in diastolic blood pressure was completely attenuated in the presence of phenoxybenzamine (Fig. 3B). Under control conditions, moxonidine induced a dose-dependent increase of diastolic blood pressure in pithed SHR compared with controls. The maximal increase was 86 ± 15 mm Hg after 1.8 mg/kg moxonidine. This increase was abolished by phenoxybenzamine.

Influence of Efaroxan and Idazoxan on the Stimulated Noradrenaline Overflow. In the presence of phenoxybenzamine (10 mg/kg), the noradrenaline concentration after preganglionic electrical stimulation was 454 ± 40 pg/ml; this was increased 3.7-, 4.7-, and 6.1-fold after efaroxan at 0.1, 1, and 3 mg/kg, respectively (Fig. 4). Idazoxan at 0.01 and 0.1 mg/kg had similar effects on stimulated noradrenaline plasma concentrations were increased by respective factors of 2 and 3 compared with phenoxybenzamine controls (Fig. 4). The stimulation-evoked increase in diastolic blood pressure in the presence of phenoxybenzamine was 4.4 ± 2.7 mm Hg, and did not change in the presence of efaroxan or idazoxan. Heart rate (417 beats/min in the absence of efaroxan and this was markedly reduced by moxonidine compared with controls (Fig. 5A). SHR serving as time controls (pre-treated with phenoxybenzamine and efaroxan, but not with moxonidine) showed almost constant noradrenaline plasma concentrations at control levels over the four stimulation periods (data not shown). When the efaroxan dosage was enhanced by a factor of 10 to 1 mg/kg, the curve depicting the moxonidine-evoked reduction of plasma noradrenaline concentration was shifted upward because values differed significantly from those achieved after low-dosage efaroxan pre-treatment (Fig. 5A). Blood pressure in all animals included in
this protocol was not affected by efaroxan and moxonidine (Fig. 5B).

Noradrenaline plasma concentrations after pretreatment with idazoxan (0.01 mg/kg) and phenoxybenzamine (10 mg/kg) and electrical stimulation were 905 ± 110 pg/ml (Fig. 6A). These plasma noradrenaline levels were not altered in time-matched controls during four stimulation periods within a total period of 80 min (data not shown). However, noradrenaline plasma concentrations were reduced by moxonidine to below 200 pg/ml (Fig. 6A). Similar to efaroxan experiments, the curves for moxonidine-induced reduction of noradrenaline plasma levels were shifted upwards when the idazoxan dosage (0.1 mg/kg) was increased (Fig. 6A). The blood pressure response also remained unaffected by idazoxan and/or moxonidine in these experiments (Fig. 6B).

Influence of Agmatine on the Stimulated Noradrenaline Overflow. The last set of experiments on electrically stimulated noradrenaline release in pithed SHR was performed using agmatine. In desipramine-free animals, agmatine (33 mg/kg) did not influence noradrenaline plasma concentra-
concentrations on its own (187 ± 35 pg/ml; data not shown), but did abolish the stimulated blood pressure increase (59.1 ± 6.3 mm Hg). In contrast, with desipramine/phenoxybenzamine (0.5/10 mg/kg) pretreated pithed SHR, the electrically stimulated plasma noradrenaline concentrations were elevated by a factor of 4.8 compared with basal levels (585 ± 47 pg/ml). Under the same conditions, additional agmatine (6 and 60 mg/kg) significantly reduced the stimulated noradrenaline plasma concentrations by 36 and 51%, respectively (Fig. 7A). Blood pressure in controls was moderately decreased by phenoxybenzamine pretreatment and was not affected by agmatine at either dose applied (Fig. 7B). Heart rate was increased and not affected by agmatine (Fig. 7C). Using desipramine (0.5 mg/kg) and AGN192403 (10 mg/kg), the basal noradrenaline plasma concentrations (151 ± 21 pg/ml) were approximately one-fourth of those seen with phenoxybenzamine pretreatment (Fig. 7A). Preganglionic stimulation increased noradrenaline overflow by a factor of 3, resulting in stimulated noradrenaline plasma levels that were only 15 to 20% of those observed with phenoxybenzamine pretreatment. In contrast to the phenoxybenzamine experiments, stimulated plasma noradrenaline concentrations were unaffected by agmatine at both applied doses (Fig. 7A). Stimulation-evoked blood pressure increased dramatically in response to AGN192403 pretreatment (ca. 100 mm Hg), which was reduced tendentiously by low-dose, but significantly (73%) by high-dose agmatine (Fig. 7B). Similar to phenoxybenzamine treatment, the stimulation-dependent increase in heart rate was not affected by AGN192403 (Fig. 7C).

**Discussion**

**Verification of an Effective α₂-Blockade.** To support the concept that imidazoline binding sites play an important role in addition to α₂-adrenoceptor in the control of sympathetic neurotransmission, experiments on noradrenaline overflow were performed in pithed SHR. From a methodological point of view, the efficacy of presynaptic adrenoceptor blockade with phenoxybenzamine was established in preliminary studies. The adequacy of α₂-blockade was clearly dem-

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**Fig. 5.** Influence of moxonidine on electrically (10 V, 0.5 Hz, 1 ms, and 3 min) stimulated plasma noradrenaline concentrations (PNA; A) and the stimulation-evoked elevation of diastolic blood pressure (DBP; B) in pithed SHR after treatment with efaroxan (○, 0.1 mg/kg; ▲, 1 mg/kg) and phenoxybenzamine (10 mg/kg). Values are expressed as means ± S.E.M. of 5 to 10 experiments. *p < 0.05, compared with moxonidine-free controls; †, p < 0.05, compared with low-dose (0.1 mg/kg) efaroxan.

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**Fig. 4.** Influence of efaroxan (○) and idazoxan (●) on the stimulation-dependent (10 V, 0.5 Hz, 1 ms, and 3 min) plasma noradrenaline concentrations (PNA) in pithed SHR that were pretreated with desipramine (0.5 mg/kg) and phenoxybenzamine (10 mg/kg). Values are expressed as means ± S.E.M. of 5 to 10 experiments. *p < 0.05, versus control.
onstrated in our study, because noradrenaline overflow was not enhanced any further when phenoxybenzamine concentrations were applied. In addition, the absence of any stimulation-evoked elevation of blood pressure after 10 mg/kg phenoxybenzamine pretreatment is considered as evidence for a complete blockade of all \( \alpha_2 \)-adrenoceptors, even though an increase in blood pressure due to noradrenaline release is attributed primarily to \( \alpha_1 \)-adrenoceptors and postsynaptically located \( \alpha_2 \)-adrenoceptors. Finally, moxonidine as an \( \alpha_2 \)-agonist did not increase blood pressure in the presence of phenoxybenzamine, which confirms the assumption that all \( \alpha_2 \)-adrenoceptors were blocked by 10 mg/kg phenoxybenzamine. A limitation associated with using phenoxybenzamine is that some consider it as an inhibitor for imidazoline binding sites (Molderings et al., 1991). However, we feel convinced that phenoxybenzamine does not occupy all imidazoline binding sites, because the mixed \( \alpha_2 /I_1 \)-antagonists efaroxan and idazoxan (Bock et al., 1999), which both counteract the hypotensive effects of moxonidine and rilmenidine (Ernsberger et al., 1990; Haxhiu et al., 1994), could reveal effects opposite to those of moxonidine. Continuing on with the moxonidine results, efaroxan as well as idazoxan increased noradrenaline overflow dose dependently during \( \alpha_2 \)-adrenoceptor blockade, strengthening the idea of antagonism between efaroxan and moxonidine at \( I_1 \)-binding sites regarding noradrenaline release. Such a picture regarding noradrenaline release is paralleled when focusing on presynaptic \( \alpha_2 \)-adrenoceptors: a stimulation for example by the agonist moxonidine causes a decrease, whereas an inhibition by rauwolscine or phenoxybenzamine increases noradrenaline release (Häuser et al., 1995). However, our findings differ from in vitro findings in that the electrically evoked \(^{[3]H} \text{noradrenaline} \) overflow in the presence of phenoxybenzamine or rauwolscine was either diminished (Göthert and Molderings, 1991; Molderings et al., 1997) or remained unchanged (Molderings and Göthert, 1998), a
fact suggesting different modes/sites of action in vivo and in vitro situations (see above for discussion).

Consistent with the perplexing findings with moxonidine and efaroxan regarding noradrenaline overflow, moxonidine markedly diminished the efaroxan- or idazoxan-evoked increases in stimulated plasma noradrenaline concentration even under $\alpha_2$-blockade (Figs. 5A and 6A). The specificity of this observation is emphasized by the fact that the moxonidine-induced reduction of stimulated noradrenaline overflow was affected differently by using two doses of efaroxan and idazoxan. This reconfirms the proposed antagonism between moxonidine and idazoxan or efaroxan concerning their actions at I1-binding sites (as suggested by others) and its regulating influence regarding noradrenaline release (Chu et al., 1997) or central blood pressure control (Ernsberger et al., 1990; Haxhiu et al., 1994).

To strengthen the putative role of imidazoline binding sites in noradrenaline release in our in vivo model, agmatine, an endogenous ligand for imidazoline binding sites (Li et al., 1994; Raasch et al., 2001), was investigated regarding its potency in regulating noradrenaline overflow. Electrical stimulation caused an elevation of plasma noradrenaline in phenoxybenzamine- or AGN192403-pretreated animals, and which was reflected in both experiments as an increase in heart rate. The magnitude of noradrenaline overflow in the presence of AGN192403 was clearly lower than that induced by $\alpha_2$-blockade. However, this is consistent with other findings (Munk et al., 1996), where AGN192403 was first characterized as an I1 ligand without any intrinsic activity. Noradrenaline overflow was markedly decreased by agmatine when $\alpha_2$-adrenoceptors were blocked, clearly emphasizing the relevancy of imidazoline binding sites. Confirming this mode of action, noradrenaline overflow was not affected by agmatine in the presence of AGN192403, because imidazoline binding sites were blocked and agmatine itself only had a low affinity toward $\alpha_2$-adrenoceptors (Li et al., 1994). After injection of AGN192403, a pronounced stimulation-evoked blood pressure increase could be observed, which was blunted by phenoxybenzamine due to $\alpha_2$-blockade. In AGN192403-treated animals, agmatine reduced the stimulation-dependent blood pressure increase only at a high concentration, probably as a consequence of an interaction with a vascular $I_3$-binding site that was recently shown to regulate vasoconstriction (Minyan et al., 2001). Our findings regarding agmatine’s influence on noradrenaline overflow reinforce the I1-binding site-mediated mechanism.

**Probable Mechanisms Underlying the Changes in Stimulated Noradrenaline Overflow.** Because our results clearly reveal an imidazoline binding site-dependent mechanism for regulating the stimulated noradrenaline overflow, it remained to be seen what imidazoline binding site subtype was involved in noradrenaline release. From our data on moxonidine, efaroxan, and AGN192403, we have clear evidence that I1-binding sites might be involved. This conflicts sharply with in vitro findings, whereby the presynaptically located non-I1/ non-I2-imidazoline binding site was characterized as regulating the release of noradrenaline as an autoreceptor (Molderings et al., 1991; Molderings and Göthert, 1995). This discrepancy indicates that the non-I1/ non-I2-binding sites are presumably not involved in mediating the in vivo observations seen in this study, a hypothesis which is also confirmed by effects seen with efaroxan and

![Fig. 7. Influence of agmatine on plasma noradrenaline concentration (PNA; A), diastolic blood pressure (DBP; B), and heart rate (C) in the presence (filled symbols) or absence (open symbols) of preganglionic stimulation (20 V, 0.5 Hz, 1 ms, and 0.5 min) in pithed SHR, which were pretreated either with desipramine/phenoxybenzamine (0.5/10 mg/kg; circles) or desipramine/AGN192403 (0.5/10 mg/kg; squares). Values are expressed as means ± S.E.M. of 8 to 10 experiments. *p < 0.05, compared with unstimulated agmatine-free controls; †p < 0.05, compared with stimulated agmatine-free controls.
idazoxan, which were classified as agonists for the non-I₁-I₂-binding site by Göthert et al. (Göthert and Molderings, 1991; Molderings et al., 1997), but which showed antagonistic features in our experiments and elsewhere (Chu et al., 1997).

So where are the I₁-binding sites that seem to be involved located? First, a ganglionic effect can be hypothesized, because I₁-binding sites are present at the cell bodies of sympathetic ganglia and adrenal medulla (Molderings et al., 1993). In addition, the observed effects of agmatine may also be due to a ganglionic effect, because 1) agmatine suppresses the nicotinic-cholinergic transmission in sympathetic ganglia due to a blockade of nicotine at the nicotinic acetylcholine (nACh) receptor (Quik, 1985; Loring, 1990) and because 2) it was shown that ganglionic excitation contributes markedly to catecholamine release in a whole animal model (Dendarower et al., 2002).

However, some results have characterized the I₁-binding sites as an excitatory binding site, because it releases atrial natriuretic peptide (Gutkowski et al., 1997; Mukhammad-Daher et al., 1997) and prostaglandins (Ernsberger et al., 1995). If this is right, activation of such receptors would evoke an increase in noradrenaline release rather than a decrease as was found in this study. In view of this, another hypothetical mechanism may involve an effect on noradrenaline release mediated via I₁-binding sites. Because stimulation of ganglionic I₁-binding sites releases prostaglandins (Ernsberger et al., 1995) and histamine (Molderings et al., 1999b), which were both shown to diminish noradrenaline release from the sympathetic nerves of cardiovascular and other tissues (Starke and Montel, 1973; Levi and Smith, 2000), it is likely that agmatine reduces noradrenaline overflow via such an indirect mechanism. Finally, nicotine was shown to increase catecholamine release by activating nACh receptors localized on peripheral postganglionic sympathetic nerve endings and in the adrenal medulla (Anden et al., 1986). It therefore seems feasible that agmatine reduces ganglionic, cholinergically evoked noradrenaline release (Yokotani et al., 2000), because agmatine inhibits nicotinic-cholinergic transmission in sympathetic ganglia (Loring, 1990). Clonidine’s potency in inhibiting an I₁-binding site-mediated activation of nicotinic channels confirms this hypothesis (Musgrave et al., 1995). However, all these indirect mechanisms must be confirmed/excluded by further studies involving H₂ and nACh receptor antagonists or cyclooxygenase inhibitors.

In summary, we have demonstrated in vivo that imidazolineline binding sites participate in noradrenaline release whereby our data indicate an I₁-binding site-mediated mechanism. Whether these I₁-binding sites are located presynaptically on the sympathetic nerve terminals seems doubtful considering findings from isolated preparation experiments. Whether an interaction with the ganglionic cholinergic, the histaminergic, or the prostaglandin system contributes to the I₁-binding site-mediated noradrenaline release needs to be clarified in future studies.

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