Pre- and postsynaptic studies concerning the interaction between the renin angiotensin system and the sympathetic nervous system
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Characterisation of a modified approach to the study of sympathetic neurotransmission and its presynaptic modulation in the isolated rabbit thoracic aorta
Introduction

The sympathetic nervous system is one of the major drug targets in cardiovascular disease. In order to investigate the physiology and pathophysiology of this target, and to identify drugs suitable as therapeutics, numerous methods, both in vivo and in vitro, have been introduced. In addition to various aspects (such as central regulation, ganglionic transmission and electrical propagation within the nerves), the transmitter release from the varicosities of the postganglionic sympathetic neuron and its effects on the postsynaptic membrane of the target organ has received considerable attention.

While the postsynaptic effects of sympathetic neurotransmitters can be investigated easily, e.g. by the addition of suitable receptor ligands under readily controlled conditions, the quantification of the transmitter release is not as simple. First of all a standard stimulus, either chemical, e.g. by the noradrenaline-releasing compound tyramine or, more common, electrically, e.g. by field stimulation, has to be applied. The quantification of noradrenaline release can be based on its effect on the target organ or by direct detection of the amount released. Direct detection can be achieved by high performance liquid chromatography (HPLC) in otherwise untreated preparations or by the detection of β-radiation released by preparations preincubated with tracer amounts of \([\text{H}]\)-noradrenaline. The latter method does not require sophisticated equipment. In addition the detection limit is superior to HPLC, which makes it more suitable for the investigation of small samples. The spillover of \([\text{H}]\)-noradrenaline has therefore become a standard method for quantification of transmitter release.

Su and Bevan\(^1\) described a technique to investigate induced sympathetic outflow in a small section of rabbit pulmonary artery. They combined the superfusion technique of Gaddum\(^2\) combined with the transmural stimulation procedure of Paterson\(^3\). The superfluent was fractionally collected and \([\text{H}]\)-noradrenaline spillover quantified as radioactivity in the sample.

In the recent past small modifications concerning this spillover method were introduced, e.g. by Storgaard and Nedergaard\(^4\) who developed a method in which superfusion was replaced by a rapid total exchange of the physiological salt solution (PSS) of an organ bath in which the preparation was submerged.

Although the technique of combined superfusion and transmural stimulation, as described by Su and Bevan\(^1\) and Storgaard and Nedergaard\(^4\), has been validated and frequently used, this approach has several methodological problems which limit its practical application and question the accuracy of the results which can be obtained. The main problem of the superfusion technique is the difficulty in establishing control of the physico-chemical conditions in the
immediate vicinity of the preparation, i.e., the local temperature and the local partial pressure for oxygen or carbon dioxide at the tip of the cannula that delivers the medium to the preparation. If not performed in a climate controlled and air current-free surrounding, the temperature of the superfusate, which runs along the tissue, will constantly drop, resulting in a gradient from the upper to the lower end of the preparation. Furthermore, if a HCO₃⁻-containing PSS is used, the pH of the PSS will increase due to a decreasing partial pressure of CO₂. In addition, the pH can be influenced by the tissue itself, since only a small volume of PSS is in contact with the tissue during superfusion and therefore the buffer capacity is minute.

Another major prerequisite of the superfusion and spillover approach is a constant and undisturbed contact between the tissue and the PSS into which the noradrenaline is released and diluted before it is finally quantified. The superfusion technique assumes (and is dependent on) a constant flow of PSS, and complete coverage the entire tissue surface. Owing to variation on the shape and size of individual preparations, this might not always be achieved.

Mechanical stress is another factor that exerts a disturbing influence. Local air currents for instance are likely to introduce an inconsistent stretch or even a repositioning of the tissue between the electrodes, thereby possibly altering sympathetic outflow. This mechanical irritation holds also true for the adjusted model of Storgaard and Nedergaard⁴, in which the tissue baths are rapidly emptied, thereby initiating intermittent vibration.

In addition to these issues, the automatic pump-systems that are necessary to properly superfuse the tissue require space and accurate handling, and intra-experimental replacement of syringes containing the compounds to be studied is an additional inconvenience. Furthermore, sampling of the radioactive superfusate can offer several practical problems, such as the contamination or a malfunction of the automatic vial rotator and inaccurate positioning of vials under the superfusion set-up.

All of the aforementioned problems indicate that modification and/or improvement of the existing techniques are desirable. For this reason we developed a modified experimental procedure to study stimulation-induced sympathetic outflow. The characteristics of the standard transmural stimulation procedure were not changed in this model, but we adjusted the superfusion technique. The artery segments used were not subjected to superfusion with PSS, but were permanently submerged in an organ bath set-up containing a fixed volume of PSS, providing the reliable control of the experimental conditions that characterizes this setup. In the presence of blockers of the most important elimination mechanisms (uptake 1 and 2), as well as blockers of the presynaptic α₂-adrenoceptors responsible for negative feedback, the actual transmitter release is reflected by the incremental accumulation of tritium in the organ bath medium.
The interactions between the renin-angiotensin-aldosterone-system (RAAS) and the sympathetic nervous system (SNS) have been investigated extensively. A complex and important role of the SNS in the pathogenesis and maintenance of cardiovascular diseases like hypertension\(^5\) and congestive heart failure\(^6\)\(^8\) is widely accepted. The same holds true for the renin-angiotensin-aldosterone system. In various cardiovascular diseases angiotensin II, the main effector hormone of the renin angiotensin cascade, induces various detrimental effects like direct vasoconstriction of vascular smooth muscle\(^9\);\(^10\), enhanced release of aldosterone from the adrenal cortex\(^11\) and cardiac and vascular hypertrophy\(^1\). In addition, angiotensin II is known to enhance sympathetic neurotransmission, and this can be demonstrated by facilitation of EFS-evoked transmitter release\(^13\);\(^16\). The potentiating effects between the RAAS and the SNS appear to be relevant in patients suffering from hypertension and congestive heart failure. For this and other reasons we characterised our modified approach by examining the actions of angiotensin II, as well as several other substances.

**Material & Methods**

**Rabbit isolated thoracic aorta preparations**

Experiments were performed with thoracic aortic rings taken from New Zealand White rabbits of either sex, weight 2000-2800g, and obtained from the Common Animal Institute Amsterdam (GDIA). The committee for Animal Experiments of the Academic Medical Center Amsterdam has approved the experimental protocol.

The rabbits were anaesthetized with Hypnorm (fentanyl/fluanison 2.5 mg/kg, i.p.) and sacrificed with pentobarbital (Nembutal 30 mg/kg, i.v.). Before pentobarbital injection the rabbits were given heparin 875IE/kg i.v. The thoracic cavity was opened and, after removal of the heart and lungs, the thoracic aorta was dissected free from its connective tissue and transferred to PPS which was oxygenated by carbogen (95% O\(_2\) + 5% CO\(_2\)) and kept at room temperature. Rings of thoracic aorta were prepared and placed in an organ bath set-up.

The PPS was composed as follows (mmol/l): NaCl 118, Na\(_2\)HPO\(_4\) 1.2, NaHCO\(_3\) 25, KCl 4.7, CaCl\(_2\) 1.6, MgSO\(_4\) 1.2, and glucose 11.0. Ascorbic acid (0.3) and Na\(_2\)EDTA (0.03) were added to prevent oxidation of noradrenaline.
Radiolabelling of noradrenergic transmitter stores

In order to label their noradrenergic transmitter stores, the rings were incubated for 45 min in 2.0 ml of PPS containing 0.1 μmol/l 1-[7,8-^3^H]-noradrenaline (specific activity 28.8 to 52.0 Ci/mmol) in a 5 ml glass-jacketed organ bath. The PPS was continuously bubbled with carbogen and maintained at a temperature of 37°C.

After the incubation period the rings were washed with [H]-noradrenaline-free PSS (10-x 2 ml and 4-x 5 ml) to remove superficially bound, non-neuronal radioactivity before the experimental procedures were started. The organ bath contained 20 ml PSS. Desipramine (0.6 μmol/l) and corticosterone (40 μmol/l) were added in order to block uptake-1 and uptake-2 (respectively) of released [H]-noradrenaline. Yohimbine (1 μmol/l) was added to the PSS to exclude any α₂-adrenergic auto-inhibitory effects of [H]-noradrenaline release. The preparations were then mounted vertically between platinum wire electrodes (2 cm) placed along either side of the preparations in a 25 ml organ bath and subjected to a tension of 0.5 g.

In order to remove superficially bound, non-neuronally released radioactivity the aortic rings were equilibrated for in total 48 min. After an initial period of 18 min the preparations were subjected to a 2 min period of electrical field stimulation (EFS) with a train of 3 ms rectangular bipolar wave pulses of 150 mA, at a frequency of 2 Hz. (S₁) (Danish Myo Technology Current Stimulator, model CS 200). This ‘priming’ stimulation has proven to increase the reliability and stability of the subsequent basal and EFS-induced [H]-noradrenaline spillover.

Stimulation of intrinsic sympathetic nerves

After the equilibration period the aortic preparations were subjected to two periods of electrical field stimulation (trains of 2 min, 3 ms, 150 mA, 2Hz). The first period of stimulation (S₁) was applied directly after the equilibration period of 48 min and the tritium outflow thus evoked was taken as control value. Subsequently, a second period (S₂) was applied 24 min after S₁. The ratio between S₂ and S₁ was used to quantify the influence exerted by the drugs to be investigated.

Measurement of tritium outflow

Samples of 0.5 ml were repeatedly taken from the organ bath starting at 36 min after washout. Since the organ bath medium was not changed on the one hand and its total volume decreased stepwise by the sampling on the other hand, the actual tritium outflow could be obtained by calculating the incremental accumulation in each sample corrected for the reduced volume. The mean basal tritium efflux/min preceding the stimulation periods S₁ and S₂ was determined as the mean outflow/min of tritium in two 6 min samples prior to each period of stimulation. For S₁,
we subtracted the radioactivity (DPM) measured in the sample taken at t=36 min from that of t=42 min and the radioactivity measured in the sample taken at t=42 min from that of the sample at t=48 min. Hence, we could determine the outflow/min of radioactivity during two 6 min time intervals prior to $S_1$. Basal outflow was determined by averaging these values. An equivalent procedure was used for $S_2$.

The release/min evoked by EFS ($S_1$ and $S_2$, 2 min samples) was calculated by subtracting the corresponding mean basal efflux/min from the apparent EFS-evoked efflux/min.

At the end of the experiment the remaining radioactivity of the tissue was measured. By adding the total released tritium to this value, the initial content of tritium was calculated. The effect of EFS on the release could then be expressed as a fraction of the total tissue content present at the time at which the EFS was applied or 'fractional release' of radioactivity ($FR_1$ and $FR_2$).

Accordingly, the effects of pharmacological interventions are expressed as the ratio $FR_2/FR_1$.

### Experiment 1 - EFS-induced spillover of $[^3]$H-noradrenaline

In this experiment we quantitatively determined the effects of electrical field stimulation on tritium outflow. EFS ($S_1$) was applied directly after the equilibration period of 48 min and the evoked outflow was compared with the basal tritium outflow. Furthermore, a second stimulation period ($S_2$) was applied in order to quantify the ratio between tritium spillover evoked by consecutive stimulations. The ratio $FR_2/FR_1$ was used to indicate the change in EFS-evoked spillover.

### Experiment 2 - EFS and sympathetic nervous system activity

To assess whether the EFS-evoked tritium spillover is of neuronal origin we tested the influence of the sodium channel blocker, tetrodotoxin (1 μM), the selective blocker of transmitter release from noradrenergic neurons, guanethidine (10 μM), and the selective N-type calcium channel blocker, ω-conotoxin GIVA (0.3 μM). Drugs were added to the PSS twenty min prior to $S_2$. The ratio $FR_2/FR_1$ was used to indicate the effects of the compounds to be studied.

### Experiment 3 - Influence of angiotensin II

To investigate the effect of angiotensin II on noradrenaline release we established the influence of angiotensin II (1 nM) on EFS-evoked tritium overflow. Angiotensin II was added to the PSS 2.5 min prior to $S_2$. The ratio $FR_2/FR_1$ was used to indicate the effect of angiotensin II.
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Detection of tritium in the samples and tissue
After the experiment the tissues were kept overnight in 2 ml of 0.5 M quaternary ammonium hydroxide dissolved in toluene (Soluene, Packard). Radioactivity was measured by liquid scintillation counting (Tri Carb 2900TR, Packard) in 20 ml aliquots (with either samples or tissue) after addition of 5 ml of the scintillation mixture (Ultima Gold, Packard). Corrections for counting efficiency were made by external automatic standardization.

Drugs and chemicals
Desipramine HCl, yohimbine HCl tetrodotoxin, guanethidine monophosphate and ω-conotoxin GVIA (all Sigma, St. Louis, USA) were dissolved in distilled water. Corticosterone (Bufa, Uitgeest, The Netherlands) was dissolved in DMSO. Stock solutions of desipramine (0.6 mM), yohimbine (1 mM) and corticosterone (40 mM) were further diluted with PSS.
Angiotensin II (Bachem, Bubendorf, Switzerland, synthetic human sequence) was dissolved in distilled water. Stock solutions of angiotensin II (0.1 nM) were stored in 50 µl aliquots at -20°C.
Tritiated levo-[7,8-3H]-noradrenaline (Amersham Pharmacia Biotech, Little Chalfont, England) had a specific radioactivity of 28.8 Ci/mmol and a radioactive concentration of 1.0 mCi/ml. Soluene and Ultima Gold solutions (Packard, The Netherlands).

Statistical analysis
All data are expressed as means ± S.E.M. An ANOVA followed by Bonferroni’s multiple comparison test was used for multiple comparisons between groups. Differences at p<0.05 were considered to indicate statistical significance.
Results

Experiment 1 - EFS-induced spillover of \(^{3}\text{H}\)-noradrenaline

In control experiments, no time-dependent change of the basal tritium efflux was observed, before or during the two periods of stimulation. The first electrical field stimulation \((S_1)\) resulted in a significant increase in the tritium efflux by approximately a factor of 4.2 compared to the resting efflux \((8339 \pm 1168 \text{ vs } 1979 \pm 139 \text{ DPM/min}; n=8, p<0.05)\) (figure 1A). Table 1 shows the mean tritium content of the aortic ring preparations at the start of the experiments in addition to (the absolute value of) \(FR_0\). The second period of stimulation \((S_2)\) resulted in a slightly lower absolute tritium spillover when compared to the first one \((S_1)\). However, when related to the absolute amount of tritium present in the tissue at the onset of the corresponding collection period (fractional release) the response to the two periods of stimulation was identical as indicated by the ratio \(FR_2/FR_1\), that is not different from unity \((0.99 \pm 0.03, n=8)\).

Since the bath medium was not changed the tritium amounted in the medium progressively (figure 1B, typical example). In this graph the EFS-evoked spillover is reflected by an increase in the slope of the line representing the accumulation versus time.

**Figure 1A.** Tritium overflow evoked by the first \((S_1)\) and the second \((S_2)\) period of electrical field stimulation in isolated rabbit thoracic aortic rings \((n=8)\). Tritium outflow (expressed as DPM/min) is plotted against time. Asterisk indicates \(p<0.05\) compared with basal outflow \((\text{ANOVA followed by Bonferroni's multiple comparisons test's})\).
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Figure 1B. Typical example of accumulated tritium amount during the experiment, corrected for volume. The amount of accumulated tritium (expressed as DPM) was plotted against time. Note the increased accumulation during the first and second period of electrical field stimulation.

Table 1. Absolute values of radioactivity (DPM) contained by ring preparations from rabbit isolated thoracic aorta at the start of the experiments in addition to the $FR_{p}$-values (absolute and as percentage of the amount present in the ring preparations at $S_{1}$) of control and treatment groups. See text for details of drug exposure. Values are shown as means ± SEM (n=5-8).

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>total tritium content (D.P.M)</th>
<th>absolute value $FR_{p}$ (D.P.M)</th>
<th>$FR_{p}$ (% total at $S_{1}$)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>9.48 ± 0.95 * 10^5</td>
<td>1.64 ± 0.28 * 10^4</td>
<td>1.94 ± 0.42</td>
<td>8</td>
</tr>
<tr>
<td>tetrodotoxin (1 μM)</td>
<td>9.73 ± 0.87 * 10^5</td>
<td>1.67 ± 0.31 * 10^4</td>
<td>2.03 ± 0.38</td>
<td>6</td>
</tr>
<tr>
<td>α-conotoxin GVI A (0.3 μM)</td>
<td>9.21 ± 0.83 * 10^5</td>
<td>1.41 ± 0.21 * 10^4</td>
<td>1.63 ± 0.21</td>
<td>6</td>
</tr>
<tr>
<td>guanethidine (10 μM)</td>
<td>9.89 ± 0.96 * 10^5</td>
<td>1.70 ± 0.29 * 10^4</td>
<td>2.15 ± 0.47</td>
<td>5</td>
</tr>
<tr>
<td>angiotensin II (0.1 μM)</td>
<td>9.08 ± 0.92 * 10^5</td>
<td>1.37 ± 0.18 * 10^4</td>
<td>1.51 ± 0.15</td>
<td>6</td>
</tr>
<tr>
<td>angiotensin II (1 μM)</td>
<td>9.65 ± 0.78 * 10^5</td>
<td>1.54 ± 0.26 * 10^4</td>
<td>1.98 ± 0.42</td>
<td>8</td>
</tr>
</tbody>
</table>

Experiment 2 - EFS and sympathetic nervous system activity
Tetrodotoxin (TTX) (1 μM) was added to the organ bath 20 min prior to the second stimulation period ($S_{2}$). The basal efflux between the consecutive periods of stimulation was not affected by
tetrodotoxin. However, the EFS-evoked overflow was nearly abolished in the presence by tetrodotoxin, as reflected by the ratio $FR_2/FR_1$ (0.06 ± 0.03, n=5, p<0.05 compared to control) (figure 2).

ω-Conotoxin GVIA (0.3 μM), added 20 min prior to $S_2$ to the organ bath did not influence the basal tritium efflux, but it abolished the EFS-induced overflow as reflected by the extremely low ratio $FR_2/FR_1$ (0.01 ± 0.06, n=6, p<0.05 compared to control) (figure 2). Guanethidine (10 μM) was added to the organ bath 20 min prior to $S_2$. It did not affect the basal tritium efflux, but attenuated the EFS-evoked overflow by approximately 50%, as reflected by the ratio $FR_2/FR_1$ (0.46 ± 0.07, n=5, p<0.05 compared to control) (figure 2).

**Figure 2.** Effects of different pharmacological active compounds on the ratio of EFS-evoked noradrenaline spillover ($FR_2/FR_1$) in the isolated rabbit thoracic aorta; the sodium channel blocker tetrodotoxin (1 μM), the selective N-type calcium channel blocker ω-conotoxin GVIA (0.3 μM), the anti-sympathomimetic agent guanethidine (10 μM) and the RAAS-effector hormone angiotensin II (0.1 nM and 1 nM). All agents had been added to the medium 20 min before $S_2$ was applied, except for angiotensin II, which had been added 150 seconds prior to $S_2$. Values are shown as means ± SEM (n=5-8). * indicates p<0.05 compared with control, ** indicates p<0.05 compared with angiotensin II 1 nM. (ANOVA followed by Bonferroni’s multiple comparisons test’s, n=6-8 per group)

**Experiment 3 - Influence of angiotensin II**

Angiotensin II (0.1 nM - 1 nM) was added to the medium 2.5 min before the second stimulation ($S_2$). The basal efflux of tritium was not affected by the presence of this peptide. However,
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angiotensin II, at each concentration, significantly enhanced the EFS-evoked spillover by nearly a factor 1.5 and 2, respectively; \( FR_2/FR \), of 1.43 ± 0.11 (0.1 nM) and 2.03 ± 0.11 (1 nM) (n=6-8, p<0.05, figure 2).

Discussion

The technique of combined superfusion and transmural electrical stimulation is based on experiments conducted by Gaddum\(^2\) and Paterson\(^3\). With this approach it is possible to investigate sympathetic neurotransmission at the level of the synaptic nerve terminal in isolated artery preparations.

In a modification of this widely used technique we could avoid several well-known difficulties of the superfusion setup by using a standard organ bath. After preincubation with \( ^{1}H \)-noradrenaline, the aortic ring preparations were mounted between the electrodes and submerged in fixed volume PPS.

The basal tritium efflux from sympathetically innervated tissues incubated with \( ^{1}H \)-noradrenaline represents predominantly \( ^{1}H \)-noradrenaline metabolites such as \( ^{3}H \)-DOPEG and \( ^{3}H \)-O-methylated deaminated (OMDA) metabolites. In rabbit pulmonary artery strips\(^4\) described that 67% of the basal tritium efflux consisted of OMDA and around 19% of DOPEG, whereas only 2% of the total tritium efflux consisted of unchanged \( ^{1}H \)-noradrenaline. By contrast, \( ^{1}H \)-noradrenaline predominantly accounts for the tritium outflow evoked by electrical field stimulation\(^5\)\(^\text{-}^9\). In rabbit pulmonary artery strips approximately 50% of the stimulation-induced tritium efflux consisted of \( ^{1}H \)-noradrenaline. DOPEG amounted to nearly 22%, whereas 19% of the total tritium efflux consisted of OMDA metabolites\(^7\). Accordingly, the tritium spillover evoked by electrical field stimulation reflects release of \( ^{1}H \)-noradrenaline rather than \( ^{1}H \)-metabolites of noradrenaline, as is the case for the basal efflux. Consequently, in our model we assumed EFS-evoked tritium spillover to reflect the release of tracer amounts of noradrenaline.

The EFS-evoked spillover of \( ^{1}H \)-noradrenaline might be influenced quantitatively by specific mechanisms within the synaptic cleft, such as re-uptake, degradation of neurotransmitter and autoinhibitory, \( \alpha \)-adrenoceptor-mediated attenuation of release. Indeed, several authors\(^1\(^\text{-}\)\(^3\)\(^\text{,}^6\)\(^\text{,}^8\)\(^\text{,}^9\) have reported significant augmentation of EFS-evoked \( ^{1}H \)-noradrenaline outflow in the presence of re-uptake inhibitors and \( \alpha \)-adrenoceptor antagonists. Accordingly, in order to rule out uptake 1
and uptake 2 of $[^1\text{H}]-\text{noradrenaline}$ we added desipramine and corticosterone, respectively. In addition, the selective $\alpha_2$-adrenoceptor antagonist yohimbine was added to the medium.

EFS caused approximately a 4-fold increase of $[^1\text{H}]-\text{noradrenaline}$ spillover as compared to the basal efflux (figure 1A). This marked increase is in accordance with the observed increase obtained in rabbit pulmonary artery strips$^{17}$ and in other perfused tissues.$^{31}$ The fractional release (release relative to total amount present in the tissue) of noradrenaline caused by $S_1$ and that produced by $S_2$ was reflected by a ratio $FR_2/FR$, not different from unity (0.99 ± 0.03). This value is in agreement with previous observations in many other isolated vessels preparations, such as rabbit thoracic aorta, the canine mesenteric and pulmonary arteries.$^{4}$ Hence, in our model, EFS is capable of substantially enhancing noradrenaline release.

Since under control conditions the fractional release caused by $S_2$ is identical to that of $S_1$, changes in the parameter $FR_2/FR$, can be used to analyze the influence of various pharmacologically active compounds modulating sympathetic neurotransmission in isolated rabbit thoracic aortic rings.

To assess the specificity of the EFS-evoked noradrenaline spillover we probed the involvement of the sympathetic nervous system at different levels. The sodium-channel blocker tetrodotoxin (1 $\mu\text{M}$) abolished the EFS-evoked noradrenaline spillover, whereas the basal efflux was unaffected (figure 2), presenting strong evidence for the neuronal origin of the released noradrenaline, as indicated by Su and Beran.$^1$ The selective N-type calcium channel-blocker $\omega$-conotoxin GVIA (0.3 $\mu\text{M}$) failed to influence the basal tritium efflux, whereas it abolished stimulation-evoked noradrenaline spillover (figure 2), thereby providing evidence for the vesicular origin of the released noradrenaline, as reported by Gother and Molderings.$^{23}$ The anti-sympatholytic compound guanethidine (10 $\mu\text{M}$) failed to alter the basal tritium efflux, whereas it attenuated the electrically evoked noradrenaline spillover by approximately 50% of control values (figure 2), which offers evidence for the involvement of sympathetic nerves. Endo et al.$^{17}$ and Fabiani and Story$^{21}$ reported an enhancement of resting efflux produced by guanethidine (1-3 $\mu\text{M}$), which they concluded was presumably a consequence of an indirect sympathomimetic action of guanethidine. The lack of effect of guanethidine on basal outflow in the present study may be explained by the observation that only a small percentage of the total basal tritium efflux consists of unchanged $[^1\text{H}]-\text{noradrenaline}$. Therefore, even a doubling of unchanged $[^1\text{H}]-\text{noradrenaline}$ release by guanethidine might be blunted by the co-release of $[^1\text{H}]$-NA metabolites. Nonetheless, our observation that guanethidine can attenuate the EFS-evoked noradrenaline outflow is in agreement with their findings. Accordingly, the results obtained with tetrodotoxin,
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ω-conotoxin GVIA and guanethidine indicate that in our modified model the applied EFS-procedure selectively evoked neuronal release of neurotransmitter by activation of sympathetic neurons.

Angiotensin II is known to bind and activate presynaptically located AT₁-receptors which results in a facilitation of sympathetic transmitter release\(^1\). In our experiments angiotensin II (0.1 nM - 1 nM) significantly enhanced (maximally by a factor 2, at 1 nM) the spillover evoked by electrical field stimulation, although basal tritium efflux remained unchanged (figure 2). Similar results have been obtained in previous studies. For instance, Storgaard and Nedergaard\(^3\) observed an enhancement of electrically evoked outflow by nearly 90% in isolated rabbit aortic rings (1 nM), whereas Endo et al.\(^17\) reported an increase by nearly 100% (10 nM) in rabbit pulmonary artery strips. In isolated rat caudal segments Cox et al.\(^10\) found the fraction \(F_{R,s}/F_R\) to be in the order of 1.6 in the presence of angiotensin II (1 μM). Moreover, angiotensin II (10 nM) increased the EFS-evoked noradrenaline spillover in the isolated canine mesenteric and pulmonary artery by 45% and 80%, respectively\(^22\). Recently, Shetty and DelGrande\(^26\) established an increase by nearly 60% of stimulation-evoked noradrenaline overflow in rat isolated atria. The congruent results on angiotensin II-induced enhancement of EFS-evoked noradrenaline release confirm the validity of the applied method.

In summary, the technique used in our experiments appears to be suitable to induce and to quantify the specific release of noradrenaline from sympathetic nerve endings. The influence of specific facilitating or inhibiting measures was demonstrated. Since the shape and size of the preparation are no longer determinants of outcome in this experimental approach, it may be possible to apply it to the study of neurotransmitter release in even small vessel preparations such as resistance arteries which could be mounted in special organ bath setups\(^2\).

In conclusion, the modified model described in the present communication appears to be valid for the study EFS-evoked sympathetic transmission and its pharmacological modulation.

References


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