MATTERS ARISING

Presence and physiological role of presynaptic inhibitory α_2-adrenoreceptors in guinea pig atria

IT was concluded by Angus and Korner[1] that "presynaptic α-adrenoreceptor modulation by synaptically released noradrenaline plays no part in cardiac sympathetic transmission".

Evaluation of their results and experimental methodology suggests that this criticism may be premature because there are important methodological differences between this study and several others which showed evidence for presynaptic α-adrenoreceptor modulation of cardiac neurotransmission. As the consensus of opinion supports a physiological role for presynaptic α-adrenoreceptors in the heart, it is useful to identify and examine these experimental differences in more detail.

(1) The study was carried out with guinea pig right atria in Krebs' solution which contains 1.2 mM Mg^{2+}. In these experimental conditions the resting rate[1] is only 110 beats per min while in most articles dealing with spontaneously beating guinea pig atria and using Locke's solution, which does not contain Mg^{2+}, the resting rate[2-5] is between 200 and 230 beats per min.

Angus and Korner state that "phentolamine 0.3–10 μM had no significant effect on the resting atrial rate or the dose–response curve to noradrenaline"[1]. The fact that their resting rate was already very low[1] may explain their findings.

However, the authors[1], without admitting their different resting atrial rate, presented these results as conflicting with findings by Langer *et al.*[6] of a negative chronotropic effect and attenuated chronotropic responses to exogenous noradrenaline in the presence of 31 μM phentolamine. However, the results of Langer *et al.*[6] were obtained with Locke's solution in conditions in which the resting atrial rate[6] was 210 beats per min. When repeating their experiments with Locke's solution Angus and Korner found that, as reported previously[6], even 10 μM phentolamine produced a significant negative chronotropic effect in guinea pig atria (J. A. Angus, personal communication). Angus and Korner did not determine whether 31 μM phentolamine antagonized responses of atria to exogenous noradrenaline as reported by Langer *et al.*[6].

(2) Instead of accelerans nerve stimulation[6] the experiment by Angus and Korner used field stimulation[1]; clearly, the latter is a less physiological stimulus than nerve stimulation, and this difference in methodology may be one of the reasons for their negative results. In support of the results of Langer *et al.*[6] it was recently reported that both phentolamine and yohimbine produce a significant enhancement of the inotropic responses to sympathetic stimulation in guinea pig left atria[7].

(3) Potentiation of the chronotropic responses to accelerans nerve stimulation at 0.5 Hz was reported in the presence of 0.1 μM phentolamine[6]. Using one or four pulses of field stimulation applied during the refractory period, exposure to 10 μM phentolamine failed to potentiate the chronotropic responses to field stimulation[1]. Recent experiments in which field stimulation was applied in guinea pig atria using four pulses at 2 Hz showed that exposure to 3 μM phentolamine did not significantly affect the overflow of ^{3}H-noradrenaline[8], thus confirming the observation of Angus and Korner[1]. However, when field stimulation was applied at 2 Hz using a total of 16 pulses, exposure to 3 μM phentolamine enhanced the overflow of the labelled transmitter as well as the peak chronotropic responses to stimulation[8]. Obviously, the negative feedback regulation of noradrenaline release mediated by presynaptic α_2-adrenoreceptors depends on the frequency and duration of neuronal activity. Failure to observe potentiation of the positive chronotropic responses to field stimulation in the report by Angus and Korner[1] is clearly related to the fact that they used too short a period of stimulation. These results[8] confirm the findings of Langer *et al.*[6] obtained with accelerans nerve stimulation.

It is premature to reach definitive conclusions, as did Angus and Korner, about the absence of feedback modulation of noradrenaline release based on negative results originating from inadequate experimental conditions which are restricted to very brief periods of stimulation. If these authors had explored a wide variety of frequencies of stimulation using different durations of the period of stimulation, their conclusions would have been quite the opposite, as clearly demonstrated by Story *et al.*[8].

(4) Although the presence of presynaptic inhibitory α_2-adrenoreceptors in guinea pig atria is questioned by Angus and Korner[1], they have shown subsequently that clonidine, an α_2-adrenoreceptor agonist, decreases the responses to field stimulation in this tissue[9]. In addition, clonidine reduces the inotropic response to sympathetic nerve stimulation in guinea pig left atria and this effect is antagonized by 5 μM phentolamine or 0.1 μM yohimbine (ref. 7). These results clearly support the view that presynaptic inhibitory α_2-adrenoreceptors are present in noradrenergic nerve endings of the peripheral nervous system[10-12]. These release-modulating presynaptic α_2-adrenoreceptors are definitely of pharmacological relevance in both *in vitro* and *in vivo* conditions[13,14]. The possible physiological role of presynaptic α_2-adrenoreceptors in noradrenergic neurotransmission is supported by the findings that α_2-adrenoreceptor blocking agents enhance both the electrically evoked release of noradrenaline and the end-organ responses to sympathetic nerve stimulation[10-12] provided suitable experimental conditions are used.

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ANGUS AND KORNER REPLY—In our paper[1] we tried to establish whether or not neurally released noradrenaline could activate an auto-inhibitory feedback on the sympathetic nerve terminals of the guinea pig right atrium and concluded that it did not. There were differences between our methodology and those of Langer and co-workers and we are grateful for the opportunity of discussing these.

(1) Bath solution. We used a standard bicarbonate-buffered solution for heart muscle[2-4] of *p*H 7.58 ± 0.01 ($n = 5$) bubbled with 95% O_2, 5% CO_2, compared with Langer and co-authors[5-7] who routinely use Mg^{2+}-free Locke's solution bubbled with 100% O_2. We found that this Locke's solution has a *p*H of 8.19 ± 0.06 ($n = 4$), a similar finding to Trendelenburg[8] and well outside the normal physiological range. The higher resting rate in Locke's solution of 180–210 beats per min compared with our rate of 110–150 beats per min are accounted for by the well known effect of *p*H on resting atrial rate[9,10] rather than the lack of Mg^{2+} which, parenthetically, is present in guinea pig

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