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Two mechanisms underlie the slow noradrenergic depolarization in the rat tail artery *in vitro*

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ABSTRACT

In rat tail artery, short trains of electrical stimuli evoke both ATP-mediated excitatory junction potentials (EJPs) and a slow noradrenaline (NA)-mediated depolarization (NAD). Here we have investigated the contribution of α_1- and α_2-adrenoceptors to the NAD. The α_1-adrenoceptor antagonist, prazosin (0.1 μM), and the α_2-antagonist, rauwolscine (1 μM), reduced the amplitude of the NAD and in combination these agents virtually abolished the NAD. The K_{ATP} channel blocker, glibenclamide (10 μM) abolished the α_2-adrenoceptor-mediated component of the NAD, indicating that activation of these receptors produces closure of K_{ATP} channels. The α_1-adrenoceptor-mediated component of the NAD was increased in amplitude by glibenclamide. Changes in membrane conductance were monitored by measuring the time constant of decay of EJPs (τEJP). The τEJP was increased during α_1-adrenoceptor-mediated depolarization, indicating a decrease in membrane conductance; i.e. closure of K^+ channels. Broad-spectrum K^+ channel blockers (tetraethylammonium, 4-aminopyridine, Ba^{2+}) and the TASK-1 K^+ channel blocker, anandamide (10 μM), did not reduce the α_1-adrenoceptor-mediated NAD. The α_1-adrenoceptor-mediated NAD was unaffected by the Cl^- channel blockers, 9-anthracene carboxylic acid (100 μM) and niflumic acid (10 μM) or by the non-selective cation channel blocker, SKF 96365 (10 μM). These findings indicate that the NAD is produced by activation of both α_1-and α_2-adrenoceptors. The α_2-adrenoceptor-mediated component is produced by closure of K_{ATP} channels whereas the α_1-adrenoceptor-mediated component is most likely mediated by closure of another type of K^+ channel.

1. Introduction

The mechanisms underlying depolarization of vascular smooth muscle by nerve-released noradrenaline (NA) remain largely unknown. In isolated vascular smooth muscle cells, activation of α_1-adrenoceptors has been demonstrated to produce an inward current by activating Ca^{2+}-activated chloride channels (Amedee et al., 1990; Large and Wang, 1996) and/or a non-selective increase in cation conductance that is most likely mediated by transient receptor potential (TRP)-like cation channels (see Albert and Large, 2006). Activation of Ca^{2+}-activated chloride channels by nerve-released NA acting at α_1-adrenoceptors produces a transient depolarization in guinea-pig mesenteric veins and rat iridial arterioles (Van Helden, 1991; Gould and Hill, 1996). However, to our knowledge, there is at present no evidence that activation of TRP-like cation channels by NA contributes to nerve-evoked depolarization in vascular preparations.

Stimulation of sympathetic nerves in both rat tail artery and guinea-pig mesenteric vein evokes a slow NA-mediated depolarization (NAD) that is associated with a decrease in membrane conductance, indicating closure of K^+ channels (Cassell et al., 1988; Van Helden, 1988). In tail artery, the NAD is due, at least in part, to α_2-adrenoceptor-mediated closure of K_{ATP} channels (Tan et al., 2007). Blockade of K_{ATP} channels depolarizes the smooth muscle in tail artery by about 5 mV (Tan et al., 2007). However, during ongoing nerve activity, released NA produces a maintained depolarization of ~20 mV and this is reduced by α_1-adrenoceptor blockade (Brock et al., 1997). Therefore, a membrane conductance controlled by α_1-adrenoceptors also contributes to nerve-evoked depolarization of vascular smooth muscle in the tail artery. In rat anococcygeus muscle, activation of sympathetic nerves produces a slow α_1-adrenoceptor-mediated depolarization that has been suggested to result from closure of K^+ channels (Bramich and Hirst, 1999).

In the present study, we have used intracellular recording techniques to examine the contribution of α_1- and α_2-adrenoceptors to the NAD evoked by short trains of electrical stimuli in rat tail artery. We also used changes in the time course of decay of purinergic excitatory junction potentials (EJPs) to assess the effects of activating α_1-adrenoceptors on membrane conductance and investigated the effects of various K^+, Cl^- and non-selective cation channel blockers on the NAD. Our findings demonstrate two components of the NAD; one of which is due to the activation of α_1-adrenoceptors and the other to activation of α_2-adrenoceptors. We confirmed that the α_2-

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adrenoceptor-mediated component of the NAD is produced by closure of K_{ATP} channels (Tan et al., 2007). Inhibition of K^+ channel activity also appears to underlie the α_1-adrenoceptor-mediated component of the NAD, as it was associated with a decrease in membrane conductance.

2. Methods

All experiments were performed in accordance to the National Health and Medical Research Council of Australia guidelines and were approved by the University of New South Wales Animal Care and Ethics Committee.

Male and female inbred Wistar rats (200–250 g) were anaesthetized (pentobarbitone 100 mg kg^{-1}, i.p) and killed by exsanguination. The ventral tail artery was dissected from between 2 and 6 cm distal to the base of the tail. Segments of the artery were pinned to the Sylgard (Dow–Corning) coated base of a 1 ml recording chamber and continuously perfused (4 ml min^{-1}) with physiological saline containing (mM): Na^+, 150.6; K^+, 4.7; Ca^{2+}, 2; Mg^{2+}, 1.2; Cl^-, 144.1; $H_2PO_4^-$, 1.3; HCO_3^-, 16.3; glucose, 7.8. This solution was heated to ~36.5 °C and was gassed with carbogen (95% O_2/5% CO_2). The perivascular nerves were electrically activated (1 ms pulse width, 20 V) via a suction-stimulating electrode into which the proximal end of the artery was drawn. As increasing the stimulus voltage did not increase the size of the evoked responses, these stimulus parameters were assumed to be supramaximal.

2.1. Electrophysiological recording

Intracellular recordings were made from smooth muscle cells located 1–2 mm distal from the mouth of the stimulating electrode using glass microelectrodes (120–180 MΩ) filled with 0.5 M KCl and connected to an Axoclamp 2B bridge amplifier (Axon Instruments, Inc. Foster City, CA, USA). To avoid excitatory junction potentials (EJPs) with an early fast component recorded in cells lying close to neuromuscular junctions at the adventitial–medial border, recordings were made from cells located deeper in the media in which EJPs decayed monoexponentially, reflecting the electrical behaviour of the smooth muscle syncytium (Cassell et al., 1988). Impalements were only accepted if the following criteria were satisfied: (1) the cell penetration was abrupt; (2) the membrane potential increased to a value more negative than the initial potential; (3) the membrane potential was stable. Resting membrane potentials (RMPs) were determined upon withdrawal of the microelectrode.

2.2. Assessing changes in membrane conductance

Because the smooth muscle cells are electrically coupled, it is not possible to directly determine the membrane resistance by injecting current through the recording electrode. However, during EJPs when a brief junctional current uniformly polarizes the smooth muscle, the time constant of decay of the EJP (τEJP) is similar to the membrane time constant (Cassell et al., 1988; Tan et al., 2007). As the membrane time constant is the product of membrane resistance and capacitance, an increase in τEJP most likely indicates an increase in membrane resistance (i.e. a decrease in membrane conductance) and vice versa. It is worth noting that, because the duration of junctional current underlying the EJP is much briefer than the membrane time constant, the amplitude of EJPs is little affected by changes in membrane conductance (see Tan et al., 2007).

2.3. Experimental protocols

In each preparation, recordings were made from three or four cells under control conditions and then, in most cases, the effects of the drug on EJPs and the NAD were assessed in a single-cell experiment in which both control and test recordings were made in the same impalement. During all impalements, arteries were stimulated at 1 min intervals with 5 stimuli at 1 Hz. To determine the effects on RMP, recordings from another two or three cells were obtained in the presence of the drug. In single-cell experiments where the impalement was lost <10 min after applying the drug, the mean EJP and NAD data for three cells in the presence of the drug were compared with those for the cells under control conditions.

2.4. Data analysis

Data were collected using a PowerLab recording system and the programs Scope and Chart (ADInstruments, Castle Hill, NSW, Australia). Subsequent analysis was made using Igor Pro (Wavemetrics, Lake Oswego, OR, USA). Prior to measurements of EJPs and NADs in each cell, three or four traces were averaged. The peak amplitude and τEJP of the first EJP in the train was determined. The τEJP was estimated by fitting a monoexponential function to the decay phase of the EJP. The peak amplitude and time to peak (measured from the 1st stimulus in the train) of the NAD were also measured. The data obtained in each tissue in the absence and in the presence of the drug were averaged prior to comparison. Statistical comparisons were made using Student's paired *t*-tests. Data are presented as mean ± SEM and the value n refers to the number of preparations studied. For all statistical tests, $P<0.05$ was taken to indicate a significant difference. In tissues where no drugs were added ($n=8$), there was no significant change in EJP or NAD amplitude during the experimental period (EJP $P=0.15$; NAD $P=0.19$).

2.5. Drugs

Glibenclamide, prazosin HCl, rauwolscine HCl, 4-aminopyridine (4-AP), tetraethylammonium (TEA), quinidine, niflumic acid and anthracene-9-carboxylic acid (9-AC) were supplied by Sigma Chemical Company (Castle Hill, NSW, Australia). Anandamide and SKF-96365 were supplied by Tocris Cookson Ltd (Bristol, UK). Prazosin was prepared as a 1 mM stock solution in 10% (v v^{-1}) dimethylsulphoxide (DMSO) in water and glibenclamide was prepared as a 10 mM stock solution in DMSO. In the experiments, 0.1% (v v^{-1}) DMSO was the highest concentration to which the tissues were exposed and this is without effects on RMP or the electrically evoked responses (see Tan et al., 2007). Quinidine and anandamide were prepared as 10 mM stock solutions in ethanol. All other drugs were prepared as stock solutions in water.

3. Results

3.1. General observations

The upper traces in Fig. 1A–C show typical membrane potential responses of the rat tail artery to stimulation with 5 stimuli at 1 Hz. Each stimulus evoked an EJP which last about 1 s and there was a slowly developing depolarization that peaked at ~10 s after starting stimulation and had an overall duration of about 1 min. In the absence of drugs ($n=20$), the peak amplitude of EJPs evoked by the first stimulus in the train was 8.1 ± 0.5 mV and their τEJP was 201 ± 8 ms. The NAD evoked by these trains of stimuli peaked 9.2 ± 0.3 s following the first stimulus in the train and had an amplitude of 2.7 ± 0.3 mV.

3.2. Blockade of α_1- or α_2-adrenoceptors differentially affected the NAD

Prazosin (0.1 μM, $n=6$) reduced the amplitude of the NAD measured 10 s following the start of the train by 81 ± 9% ($P<0.001$; Fig. 1A) whereas rauwolscine (1 μM, $n=8$) reduced it by 43 ± 5% ($P<0.001$; Fig. 1B). In the presence of prazosin, the remaining NAD peaked at 14.2 ± 1.2 s, while in the presence of rauwolscine, the NAD