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α_1-adrenoceptor-mediated depolarization and β-mediated hyperpolarization in cultured rat dorsal root ganglion neurones

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Abstract

The mechanism of sympathetic – sensory coupling after nerve injury is still not well understood. We have studied the changes in resting potential and excitability of sensory neurones induced by adrenergic stimulation, using whole-cell and perforated-patch recordings in cultured dorsal root ganglion neurones from normal rats. Adrenaline (1–100 μM) depolarized 18 of 39 neurones (46%) and hyperpolarized seven neurones (18%); excitability was increased and decreased, respectively. Stimulating the neurones with 10 μM phenylephrine (α_1-agonist) induced depolarization and increased excitability, while 10 μM isoprenaline (β-agonist) induced hyperpolarization and reduced excitability. We conclude that α_1- and β-receptors have opposing effects on membrane potential and excitability in cultured dorsal root ganglion neurones, and the differing effects of adrenaline can be explained by different degrees of expression of each receptor type. © 2002 Published by Elsevier Science Ireland Ltd.

Keywords: Adrenaline; Phenylephrine; Isoprenaline; Excitability; Primary sensory neurone; Axotomy; Neuropathic pain

Primary afferents become sensitive to catecholamines after nerve lesion, permitting abnormal excitation either by noradrenaline, which is released from postganglionic sympathetic terminals, or by circulating adrenaline [10]. Postganglionic sympathetic fibers sprout around large neurones in the dorsal root ganglion (DRG) after axotomy, and this may be involved in neuropathic pain [11]. However, adrenergic sensitivity can arise in injured afferents independent of sympathetic sprouting [9,15]. Sympathetic effects on injured afferents in vivo are mediated by α-adrenoreceptors [3]. Both α_1 and α_2 subtypes have been shown to be involved: in rodents after axotomy, α_2-adrenergic receptors mediate the excitatory response [1,4,16], whereas in fibers remaining uninjured after nerve lesion in primates, the α_1-subtype seems to be involved [2]. However, nerve injury may not be required for adrenergic sensitivity: adrenaline may activate intact cutaneous afferents or normal cultured DRG neurones via β-adrenergic receptors [1,8], although this is not a consistent finding [7].

While investigating adrenaline effects in DRG neurones, we found that its effect was not always excitatory: in some cases adrenaline hyperpolarized DRG neurones and reduced excitability. Here we show that in cultured DRG neurones from normal rats, hyperpolarization and reduced excitability result from β-adrenergic stimulation, while α_1-adrenergic stimulation induces depolarization and increases excitability.

Cultures of DRG neurones from adult male Wistar rats (150–250 g) were prepared essentially as previously described [14], except that capsaicin was not used and 10% horse serum (Sigma) replaced the serum-free supplement. Recordings were performed at room temperature (22–25°C) within 2–5 days from plating. The standard extracellular solution contained in mM: 140 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 glucose, 10 HEPES, adjusted to pH 7.4 (at 25°C) with 1 M NaOH, and the pipette solution: 10 NaCl, 120 KCl, 3.45 $MgCl_2$, 1 $CaCl_2$, 10 EGTA, 10 HEPES, 2 MgATP and 0.1 LiGTP, adjusted to pH 7.2 (at 25°C) with 1 M KOH. Amphotericin perforated patch recordings were made as described elsewhere [14] using a K_2SO_4-based pipette solution. Test solutions were applied by gravity with a flow of 0.5 ml/min, through a 0.8 mm diameter tip positioned 0.8 mm from the cell.

Current clamp recordings were performed with an EPC-7 amplifier (HEKA – Elektronik, Lambrecht, Germany), using borosilicate glass pipettes (GC150TF, Harvard Appa-

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ratus, Edenbridge, Kent, UK), heat-polished to a resistance of 2–7 MΩ. Stimuli were controlled and digital records captured with pClamp 5.5.1 software and an MC-DAS 1612 interface (Scientific Solutions, Mentor, OH, USA). Data were analyzed using pClamp 8 (Axon Instruments, Union City, CA, USA). All values are expressed as mean ± SD.

Cells with resting membrane potential more positive than −40 mV or more negative than −70 mV were not accepted. We classified the cells as small (mean diameter less than 32 μm) or medium (32–50 μm) and we measured the properties of the action potentials elicited during a 100 ms step depolarizing current. The parameters measured were based on Fig. 1 of ref. [5]: resting membrane potential, latency (from the beginning of the pulse to the takeoff point), time to peak (measured from the takeoff point), decay time (from the peak until the return to the baseline voltage measured at the end of the depolarizing pulse), action potential duration (time to peak + decay time), and the time to 80% recovery from afterhyperpolarization (AHP80). Resting and action potentials and responses to adrenaline, the α_1-agonist phenylephrine and the β-agonist isoprenaline did not vary systematically with cell size or recording configuration (whole-cell, $n = 35$, or perforated patch, $n = 17$), so all recordings are considered as one group. Reversibility and repeatability of the responses were also independent of recording configuration. A cell was considered to have responded to an agonist if the resting potential changed by more than 2 mV in either direction, or if either the action potential duration, latency, or AHP80 changed by more than 10%.

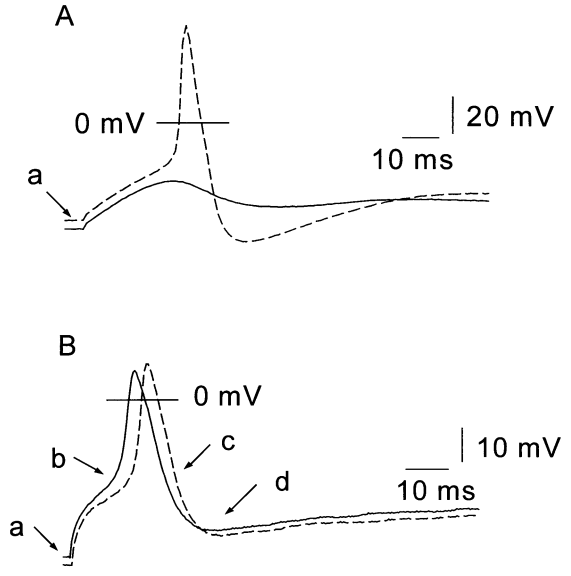

Fig. 1. Effects of adrenaline (10 μM) on resting and action potentials in DRG neurones. (A) Action potential of the same neurone in the absence (continuous line) and presence of adrenaline (dashed line). Adrenaline depolarized the cell by 4.5 mV (a) and increased excitability so that the stimulus now elicited an action potential. (B) In a different neurone, 10 μM adrenaline induced a hyperpolarization of 3.2 mV (a); an increase in latency (b); decrease in action potential duration (c); and increase in AHP80 (d).

The present study is based on 52 neurones of which 45 were classified as small (29.2 ± 3.2 μm) and seven as medium (41.1 ± 5.0 μm). In 31 of the 45 small neurones and six of seven medium neurones the injected current elicited an action potential, while in the remaining 15 neurones no action potential was elicited by the maximum current of 999 pA that the EPC-7 can produce. Resting potential was −54.5 ± 10.9 mV ($n = 52$), action potential duration 7.7 ± 3.7 ms, overshoot 37.7 ± 5.8 mV, and AHP80 22.5 ± 10.7 ms ($n = 37$ for all action potential parameters). Comparing the action potential duration for both small and medium cells with published values for sensory neurones [5], they correspond to either nociceptive neurones or a group identified in that study as unresponsive to non-noxious and noxious mechanical and thermal stimuli.

Adrenaline was applied to 39 neurones, of which 25 fired action potentials in response to depolarizing current, at a concentration of 1 [8], 10 [1,13] and 100 μM [7,18]. The higher concentrations produced no increase in the effect compared with 1 μM, suggesting that 1 μM was already a maximal concentration; responses at the three concentrations were therefore considered as a single group. Of 39 neurones, 18 were depolarized by 4.9 ± 3.6 mV (Fig. 1A), seven were hyperpolarized by 6.9 ± 3 mV (Fig. 1B) and one showed a biphasic response, a depolarization of 5.5 mV followed by a hyperpolarization of 7 mV. The remaining 13 neurones showed no significant change in resting potential, although one became markedly more excitable (threshold current decreased from 500 to 350 pA). The depolarization induced by adrenaline was not enough to trigger repetitive activity in any of the cells. The changes in membrane potential induced by adrenaline were complete after 2 min and reversible in 1–5 min.

The action potential parameters also changed in both directions. In ten of 18 neurones that were depolarized by adrenaline, the action potential duration increased by 21.7 ± 12.4% (range 10.3–54.3%) and the latency decreased by 34.8 ± 24.3% (range 11.7–76%) indicating increased excitability; in six of these neurones, the AHP80 increased by 23.5 ± 9.0% (range 16.7–38.9%) (Fig. 1A). In five of seven neurones that were hyperpolarized, action potential duration decreased by 31.9 ± 20.5% (range 10.5–57.6%), and in three of them the latency increased by 21.2 ± 14.5% (range 10.6–37.8%) indicating decreased excitability, and the AHP80 increased slightly by 10.0 ± 1.2% (range 8.7–11.0%) (Fig. 1B). Effects on action potential duration were primarily attributable to changes in the decay time. It is notable that, although action potential parameters in general changed in opposing directions in neurones that were depolarized or hyperpolarized by adrenaline, the AHP80 was prolonged by adrenaline in both groups and never shortened.

To test whether these opposing effects of adrenaline depend

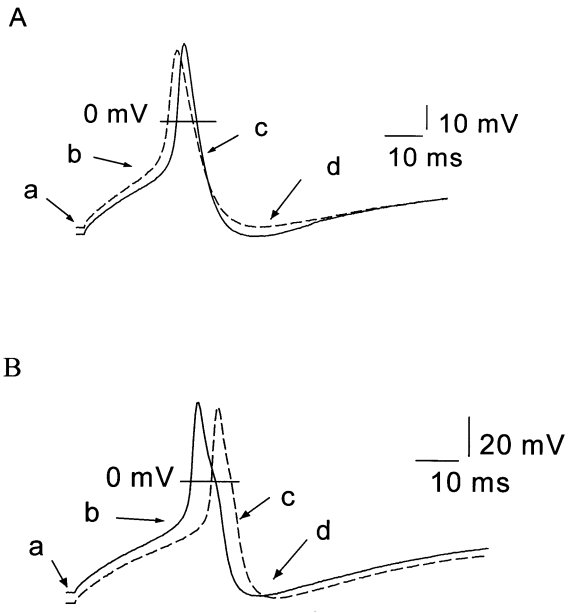

Fig. 2. Action potentials recorded in the presence of α_1- and β-adrenergic agonists. (A) 10 μM phenylephrine (dashed line) induced a depolarization of 2.8 mV (a); shortened the latency (b); increased the action potential duration (c); and increased the AHP80 (d) compared to control (continuous line). (B) 10 μM isoprenaline (dashed line) induced a hyperpolarization (a); increased the latency (b); decreased the action potential duration (c); and increased the AHP80 (d).

on the presence of different receptor subtypes, we looked at the effects of specific α_1 and β agonists on a total of 23 neurones, of which ten had previously been treated with adrenaline. We applied the α_1-agonist phenylephrine (10 μM) to ten neurones (Fig. 2A); it depolarized four of them slightly (3.3 ± 1.1 mV, range 2–4 mV) and it had no effect on the resting potential of the remaining 6 neurones. There was an increase in action potential duration (17.4 ± 2.2%, range 14.3–19.1%, $n = 4$, three of which were depolarized) and in one cell AHP80 was increased slightly (by 11.1%). Latency was decreased (43.6 ± 31.8%, range 14.8–81.2%, $n = 5$, three of which were depolarized). Three of the six cells where we applied both adrenaline and phenylephrine had been depolarized by adrenaline, and two of these were also depolarized by phenylephrine.

The β-agonist isoprenaline (10 μM) was applied to 15 neurones, of which six had been previously treated with adrenaline. Seven of 15 neurones were hyperpolarized by 6 ± 3.3 mV (Fig. 2B), and there was no change in the remaining eight neurones. The action potential duration was decreased in three hyperpolarized neurones by 30.8 ± 15.9%, (range 13.8 – 45.5%, $n = 3$), latency was increased (20.6 ± 8.6%, range 13.5–30.2%, $n = 3$) and AHP80 was increased (29.1 ± 12.9%, range 19.4–46.7%, $n = 4$, two of which were hyperpolarized). Of the six cells where we applied both adrenaline and isoprenaline, three were hyperpolarized by both (3.7 ± 1.6 mV in adrenaline, 5.6 ± 1.2 mV in isoprenaline), two were depolarized by adrenaline and hyperpolarized by isoprenaline and one did not respond to either agonist. The results are summarized in Table 1.

In this study we have shown that adrenaline has opposing effects on cultured DRG neurones. Some are depolarized and made more excitable, and the action potential duration is prolonged; these effects can be reproduced by the α_1-agonist phenylephrine. Others are hyperpolarized and made less excitable, and the action potential duration is shortened, and these effects can be reproduced by the β-agonist isoprenaline. Adrenaline prolonged the AHP80 or left it unaffected, but never shortened it; similarly, both phenylephrine and isoprenaline either prolonged the AHP80 or left it unchanged, but neither agonist shortened it. The consistency of the responses to isoprenaline or phenylephrine and the variability of responses to adrenaline can most simply be explained if α_1- or β-receptors couple to the same mechanism in all DRG neurones, with the overall response to adrenaline being determined by the presence or relative density of the receptor types in each neurone, or by the efficiency with which each receptor type couples to its effector mechanism(s).

The best characterized effect of both α- and β-adrenoceptor stimulation in the DRG is the activation of various types of voltage-gated Ca^{2+} channels [1,6]. Although these effects are probably operating, all the effects of adrenergic stimulation we report here can be explained more simply by effects on K^+ conductances active at the resting potential. Background K^+ conductances in the DRG are probably contributed largely by two-pore-domain K^+ channels, which are expressed in the DRG [12] and can be modulated in other neurone types by adrenergic stimulation [17], raising the

Table 1
Change in action potential parameters in response to adrenergic agonists[a]

Parameters	Adrenaline (*n*) (depolarized neurones)	Phenylephrine (*n*)	Adrenaline (*n*) (hyperpolarized neurones)	Isoprenaline (*n*)
Resting potential (mV)	4.9 ± 3.6 (18/39)	3.3 ± 1.1 (4/10)	− 6.9 ± 3.0 (7/39)	−6.0 ± 3.3 (7/15)
AP duration (% change)	+21.7 ± 12.4 (6/18)	+17.4 ± 2.2 (3/4)	− 31.9 ± 20.5 (5/7)	−30.8 ± 15.9 (3/7)
Latency (% change)	−34.8 ± 24.3 (10/18)	−43.6 ± 31.8 (3/4)	+ 21.2 ± 14.5 (3/7)	+20.6 ± 8.6 (3/7)
AHP80 (% change)	+23.5 ± 9 (6/18)	+11.1 (1/4)	+ 10.0 ± 1.2 (3/7)	+19.4 (2/7)

[a] All values are given as mean ± SD; *n* represents the number of cells from each group; positive values for change in resting potential indicate depolarization. AHP80 is the time to 80% recovery from afterhyperpolarization (see text).

possibility that similar mechanisms may be operating in the DRG.

There is some disagreement in the literature about the effects of adrenergic stimulation on dissociated or cultured DRG neurones from uninjured animals: two studies have shown an excitatory effect of adrenaline mediated by β-receptors [1,8], whereas another showed no effect of noradrenaline [7]. In contrast, there is clear evidence in vivo and in vitro of an excitatory effect of adrenergic stimulation after nerve injury, mediated by α-receptors [1,3,4,7]. We have shown behaviour more similar to axotomized than to normal neurones, with α-1-adrenerger stimulation increasing excitability and β-stimulation decreasing it. The only major methodological difference between this study and earlier ones in dissociated DRG neurones is the time in culture; we kept the neurones in culture for 2–5 days, while others recorded within 2–10 h [1], 24 h [8] or 2 h–2 days [7]. A longer period in culture allows more time for the effects of axotomy, an inevitable result of the preparation of dissociated DRG neurones, to manifest themselves. We therefore suggest that normal DRG neurones kept in culture for some days may behave more like injured neurones than normal ones, which may make them a useful alternative to in vivo axotomy for the study of some aspects of nerve injury.

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