PC115

A comparison of the effects of capsazepine on type I and type II slowly adapting mechanoreceptors in the rat sinus hair follicle

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There is currently significant interest in determining the molecular bases for mechanotransduction in the vertebrate (Lumpkin & Caterina, 2007). We therefore tested a broad spectrum transient receptor potential (TRP) channel antagonist in an isolated sinus hair follicle preparation in which slowly adapting type I (St I) and type II (St II) could be distinguished.
28 adult Wistar-derived rats (mean weight 335 g) were used. Sinus hair follicles with a 10 mm length of deep vibrissal nerve attached were microdissected from the whisker pad of animals killed by I.P. and I.C. 3g/kg urethane). Follicles were kept in carbogenated (bubbled medical 95% oxygen, 5% carbon dioxide) synthetic interstitial fluid (SIF). They were slit open lengthways and fixed with insect pins to a silicone elastomer Sylgard platform in a custom-made tissue bath. Capsazepine was made up in SIF and used between 10 – 200 μM. Doses were applied to the preparation at the rate of 1 ml/min for up to 20 min. The two types of units were distinguished by their characteristic static phase firing as previously described (Senok & Baumann, 1997). Statistical analyses included t tests.
Capsazepine was tested on a total of 13 St I units. Between 30 – 200 μM caused a transient increase in activity (mean±SEM 52±5 Hz to 80±8 Hz, $p < 0.01$), notably of the static component. Repeated doses resulted in clear habituation to this excitatory effect. With high concentrations 100 – 200 μM the excitatory effect was followed by a long-lasting and profound depression of all activity ($p < 0.01$). A total of 15 St II units were studied. Only doses above 50 μM had any effect, and this consisted of a uniform and long-lasting depression of all activity components (dynamic, static and spontaneous) ($p < 0.01$). In about 25% of St II units which were spontaneously active, a delayed drug effect produced an inversion of response such that ongoing firing was interrupted during the mechanical ramp stimulus. The same effect was only seen in one St I unit.
The depression by capsazepine of all activity in both St I and St II units suggest a non-selective effect on mechanoreceptor nerve endings. The excitatory effect of capsazepine that was seen only in St I units may represent an activation of Merkel cells. In another in vitro cell system, capsazepine has been found to increase intracellular calcium (Huang et al, 2006). Pharmacological manipulation (e.g. caffeine (Senok & Baumann, 1997)) which cause calcium influx in Merkel cells results in a selective increase in the static component, as seen here. In conclusion, these results using capsazepine provide weak support for a TRP channel role in St I and St II mechanotransduction. However, the excitatory effect seen in St I mechanoreceptors emphasises their difference from St II mechanoreceptors.

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Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC116

Orexin/hypocretin-A induces intracellular calcium transients in rat cultured dorsal root ganglia neurones

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The hypothalamic peptides orexin A/hypocretin-1 and Orexin B/hypocretin-2 are involved in a range of physiological functions including control of feeding and energy metabolism, sleep and arousal. Orexin fibers innervate many regions of the brain and spinal cord which include areas involved in pain processing and emerging evidence suggests that orexins modulate pain transmission. This study investigate the effects of Orexin A on intracellular calcium, ([Ca2+]i), signals in cultured rat dorsal root ganglion (DRG) neurons, with the aim of exploring possible involvement of this agent in nociceptive transmission. DRG neuronal cultures were loaded with 1 μmol Fura-2 AM and Ca2+ responses were assessed by using the fluorescent ratiometry. Fura-2 loaded DRG cultures were excited at 340 and 380 nm, and emission was recorded at 510 nm by using imaging system consisting of CCD camera coupled to an inverted microscope with a 40x (1.30 NA S Fluor, Oil) objective. [Ca2+]i changes were determined by the change in 340/380 ratio (basal-peak) was also calculated for individual DRG neurons in selected microscopic fields. All data were analyzed by using unpaired t test, with a 2-tailed P level of <.05 defining statistical significance. ORX-A caused increase in [Ca2+]i in a dose dependent manner. The ORX-A-induced [Ca2+]i responses were similar to those observed with high-K+ (30 mM). The mean 340/380 nm ratios were (baseline vs OrX-A): 0.90±0.01 vs 1.34±0.03 (1 nM OrX-A, $P<0.001$, n=21); 1.10±0.03 vs. 2.15±0.09 (10 nM OrX-A, $P<0.001$, n=21); 0.82±0.02 vs. 1.65±0.09 (100 nM OrX-A, $P<0.001$, n=18) and 0.72±0.02 vs. 1.69±0.08 (200 nM OrX-A, $P<0.001$, n=34), respectively. These results, in line with previous findings in different preparations, show that Orexin A increases intracellular calcium levels in a dose-dependent fashion in DRG neurons. We conclude that orexin-A has excitatory effects on DRG neurones, consistent with the perspective that orexin/hypocretins have a role in orchestrating reactions related to nociception, pain and temperature sense.
Keywords: Orexin A; fluorescence calcium imaging, nociception, sensory neurones

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PC117

Epac activation, altered calcium homeostasis and ventricular arrhythmogenesis in Langendorff-perfused mouse hearts

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The recently described cAMP sensor, Exchange protein directly activated by cAMP (Epac), has been implicated in distinct cAMP-dependent, protein kinase A-independent cellular signalling pathways (Bos JL, 2006). We investigated effects of Epac activation in catecholamine-induced ventricular arrhythmogenesis. In contrast to control findings (n = 20), monophasic action potentials showed spontaneous triggered activity in 2 out of 10 intrinsically beating and 5 out of 20 extrinsically-paced Langendorff-perfused murine hearts perfused with the specific Epac activator 8-pCPT-2'-O-Me-cAMP (8-CPT, 1 μM) (Christensen AE *et al.* 2003). During steady extrinsic pacing at 8 Hz, 3 out of 20 such hearts showed spontaneous ventricular tachycardia (VT). Programmed electrical stimulation provoked VT in 10 of 20 similarly treated hearts ($P < 0.001$; $n = 20$, Fisher's Exact Test). However, no statistically significant changes ($P > 0.05$, ANOVA) in left ventricular epicardial (40.7 ± 1.2 versus 44.0 ± 1.7 ms; n = 10), or endocardial action potential durations (APD_{90}) (51.8 ± 2.3 versus 51.9 ± 2.2 ms; n = 10), transmural (ΔAPD_{90}) (11.1 ± 2.6 versus 7.9 ± 2.8 ms; n = 10) or apico-basal gradients of repolarization, ventricular effective refractory periods (29.1 ± 1.7 versus 31.2 ± 2.4 ms in control and 8-CPT-treated hearts, respectively; n = 10) and APD_{90} restitution characteristics accompanied these arrhythmogenic effects. However, fluo-3 fluorescence imaging of cytosolic Ca^{2+} demonstrated alterations in Ca^{2+} homeostasis in the form of increased Ca^{2+} wave generation in both paced and resting isolated 8-CPT-treated ventricular myocytes. An independent method of Epac activation that applied 100 nM isoproterenol to stimulate beta-adrenoreceptors in parallel with protein kinase A inhibition by 2 μM H-89, was also arrhythmogenic in the whole heart and similarly altered cytosolic Ca^{2+} homeostasis. The Epac-dependent effects at both the whole heart and cellular levels were reduced by inhibition of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) with 1 μM KN-93. These findings associate VT in an intact cardiac preparation with altered cellular Ca^{2+} homeostasis and Epac activation through a CaMKII-dependent mechanism for the first time, in the absence of the altered repolarization gradients previously implicated in re-entrant arrhythmogenesis (Killeen MJ *et al.* 2007; Thomas G *et al.* 2007).

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Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC118

Is nitric oxide (NO) important in the adenosine A_{2A}-receptor-mediated vasodilatation of skeletal muscle contraction?

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During systemic hypoxia the contribution of adenosine to skeletal muscle vasodilatation is dependent on the presence of NO; NO is required for the release of adenosine from the endothelium (Edmunds *et al*. 2003) and mediates dilatation via endothelial A_1-receptors (Ray & Marshall, 2005). By contrast, skeletal muscle vasodilatation accompanying muscle contraction (exercise hyperaemia) is mediated by adenosine acting at A_{2A}-, but not A_1-receptors (Ray & Marshall, 2008). Adenosine can release NO from endothelium by acting at A_{2A}-receptors (Ray *et al*. 2002). Thus, we investigated the role of NO in exercise hyperaemia.

In three groups of rats, anaesthetized with Saffan (7-12 $mgkg^{-1}hr^{-1}$ I.V.), we recorded arterial blood pressure (ABP), femoral blood flow (FBF) and tension in the extensor digitorum longus. Isometric twitch contractions were evoked by stimulation of the sciatic nerve at 4Hz. Integral femoral vascular conductance (IntFVC) was calculated off-line. Group 1 (n=7) was the time control for, Group 2 (n=10), which received NOS inhibitor L-NAME before the third, and A_{2A}-receptor antagonist ZM241385, before the fourth contraction. Group 3 (n=12) received L-NAME before the third, the NO-donor SNAP to restore baseline FVC during the fourth and fifth contraction and ZM241385 before the fifth.

Time controls showed consistent tension and hyperaemic responses. In Group 2, baseline IntFVC was reduced by L-NAME (0.555±0.04 (mean±SEM) to 0.297±0.02CU*, ANOVA for repeated measures, p<0.001) but not by ZM241385. L-NAME reduced exercise hyperaemia (13.91±1.31 to 9.52±1.09CU*), and it was further attenuated by ZM241385 (to 5.46±1.12CU*). In Group 3, SNAP after L-NAME restored baseline IntFVC to control levels (Control: 0.702±0.09, L-NAME: 0.377±0.05*, L-NAME + SNAP: 0.616±0.09CU); ZM241385 had no further effect. Exercise hyperaemia was also restored to control levels after L-NAME by SNAP (Control: 17.10±1.18, L-NAME: 10.87±1.09*, L-NAME + SNAP: 16.99±1.38CU), and this response was further attenuated by ZM241385 (12.75±0.98CU*).

These results confirm that adenosine acting via A_{2A}-receptors contributes to exercise hyperaemia. However, they indicate