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Invited Perspective

Multiple nickel-sensitive targets elicit cardiac arrhythmia in isolated mouse hearts after pituitary adenylate cyclase-activating polypeptide-mediated chronotropy

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ABSTRACT

The pituitary adenylate cyclase-activating polypeptide (PACAP)-27 modulates various biological processes, from the cellular level to function specification. However, the cardiac actions of this neuropeptide are still under intense studies. Using control (+|+) and mice lacking (−|−) either R-type ($Ca_v2.3$) or T-type ($Ca_v3.2$) Ca^{2+} channels, we investigated the effects of PACAP-27 on cardiac activity of spontaneously beating isolated perfused hearts. Superfusion of PACAP-27 (20 nM) caused a significant increase of baseline heart frequency in $Ca_v2.3$(+|+) (156.9 ± 10.8 to 239.4 ± 23.4 bpm; $p < 0.01$) and $Ca_v2.3$(−|−) (190.3 ± 26.4 to 270.5 ± 25.8 bpm; $p < 0.05$) hearts. For $Ca_v3.2$, the heart rate was significantly increased in $Ca_v3.2$(−|−) (133.1 ± 8.5 bpm to 204.6 ± 27.9 bpm; $p < 0.05$) compared to $Ca_v3.2$(+|+) hearts (185.7 ± 11.2 bpm to 209.3 ± 22.7 bpm). While the P wave duration and QTc interval were significantly increased in $Ca_v2.3$(+|+) and $Ca_v2.3$(−|−) hearts following PACAP-27 superfusion, there was no effect in $Ca_v3.2$(+|+) and $Ca_v3.2$(−|−) hearts. The positive chronotropic effects observed in the four study groups, as well as the effect on P wave duration and QTc interval were abolished in the presence of Ni^{2+} (50 μM) and PACAP-27 (20 nM) in hearts from $Ca_v2.3$(+|+) and $Ca_v2.3$(−|−) mice. In addition to suppressing PACAP's response, Ni^{2+} also induced conduction disturbances in investigated hearts. In conclusion, the most Ni^{2+}-sensitive Ca^{2+} channels (R- and T-type) may modulate the PACAP signaling cascade during cardiac excitation in isolated mouse hearts, albeit to a lesser extent than other Ni^{2+}-sensitive targets.

1. Introduction

The pituitary adenylate cyclase-activating polypeptide (PACAP) belongs to a large family of biologically active peptides and regulates a wide range of physiological processes [1,2]. PACAP is highly conserved and is present in two bioactive forms: PACAP-38 and PACAP-27 with 38 and 27 amino acid residues respectively. PACAP(1–27) (amino acid residues 1–28 on N-terminal amidated PACAP) exhibits high homology to vasoactive intestinal peptide [3]. This peptide is widely distributed in the brain and peripheral tissues and organs [4]. In recent years, the cardiac modulatory role of PACAP has received considerable attention [5].

Previous studies have reported an important role of PACAP in cardiac functions, both *in vivo* [6] and *in vitro* [1,7–9]. These studies revealed controversial and opposite results such as positive inotropy, positive and negative chronotropy, and dromotropic effects of PACAP. The positive inotropic and chronotropic effects were attributed to direct stimulation of cardiomyocytes (CMs) [4,10], whereas bradycardia was suggested to be due to presynaptic regulation of acetylcholine release from intracardiac parasympathetic nerves [4]. In studies involving isolated guinea pig hearts, the PACAP-induced negative chronotropy was also mainly attributed to an increase in acetylcholine release from parasympathetic neurons, while tachycardia was suggested to originate from direct stimulation of sympathetic nerve terminals [6]. In addition, PACAP-27 was shown to induce chronotropy when reperfused in isolated mouse atria [1]. Taken together, the above mentioned results suggest that PACAP contributes to the regulation of cardiac function, but the mechanistic path is far from being understood. Given the function of voltage-dependent Ca^{2+} channels (VDCCs) in regulating cardiac functions, it is hypothesized that highly Ni^{2+}-sensitive VDCCs, espe-

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cially, the R- ($Ca_v2.3$) and T-types (and $Ca_v3.2$) may be involved in this process [9].

High-voltage-activated (HVA) $Ca_v2.3$ Ca^{2+} (α_1E) channels encode pharmacoresistant R-type currents ($I_{Ca,}R$) and are mainly distributed throughout the central and peripheral nervous systems [11,12]. Accumulating evidences suggest that $Ca_v2.3$ channels also contribute to, and/or regulate cardiac automaticity and rhythmicity. $Ca_v2.3$ channel transcripts, as well as the $I_{Ca,}R$ have been detected in CMs [13]. We previously revealed that intrinsic cardiac $Ca_v2.3$ channels, significantly contribute to normal regulation of ventricular conduction [13], as the ablation of $Ca_v2.3$ is associated with irregularities related to sinoatrial dysfunction. Isolated $Ca_v2.3$-deficient ($Ca_v2.3(-|-)$) mouse hearts exhibited chronic P-wave anomalies [13,14], which were reproduced upon application of Ni^{2+} in hearts from control ($Ca_v2.3(+|+)$) mice [13].

While the roles of R-type Ca^{2+} channels (RTCCs) are still being uncovered, those of T-type Ca^{2+} channels (TTCCs) ($Ca_v3.1$ and $Ca_v3.2$) are well accepted [15–17]. TTCCs are mainly prevalent in the conduction system in the adult heart where they contribute to generating pacemaker depolarization and automaticity [17]. Ablation of $Ca_v3.1$ is associated with bradycardia and slow ventricular conduction [18], whereas total deletion and Ni^{2+} inhibition of $Ca_v3.2$ channels did not affect the heart rate and ECG wave morphology, but caused recurrent coronary vasospasm [19].

Inorganic Ni^{2+} ions have been routinely used as a tool to inhibit VDCCs, although differential effects on the various subgroups are recorded. Nevertheless, multiple studies show that low concentrations of Ni^{2+} ions preferentially inhibit $Ca_v2.3$ and $Ca_v3.2$ channels [12,20,21]. The half maximal concentrations for $Ca_v2.3$ and $Ca_v3.2$ are close to each other (10–30 μM), making it difficult to specifically study $I_{Ca,}R$ in tissue co-expressing $Ca_v3.2$, such as in cardiac tissues [22].

PACAP was recently reported to induce excitation in intracardiac ganglia [9,23] and in mouse adrenal chromaffin cells [24]. This excitatory effect probably requires the recruitment of $Ca_v3.2$ [24], and/or $Ca_v2.3$ Ca^{2+} channels [9]. Taking into account the reported functions of these low and intermediate VDCCs in the heart and the implication of PACAP in cardiac function, we studied the contribution of this peptide in cardiac activity in the context of $Ca_v2.3$ or $Ca_v3.2$ ablation. Therefore, to test the hypothesis that stimulatory effect of PACAP on heart rhythm requires the expression of the highly Ni^{2+}-sensitive Ca^{2+} channels ($Ca_v2.3$ or $Ca_v3.2$), we examined the effects of PACAP in isolated hearts of control ($Ca_v2.3(+|+)$ or $Ca_v3.2(+|+)$) and transgenic ($Ca_v2.3(-|-)$ or $Ca_v3.2(-|-)$) mice.

2. Materials and methods

2.1. Animals and ethical considerations

All experiments involving $Ca_v2.3$ channels were conducted using male mice with a mixed background (C57Bl/6 and 129SvJ). For generation of $Ca_v2.3(-|-)$ mutants, the Cacna1e gene encoding $Ca_v2.3$ was disrupted *in vivo* by agouti-colored $Ca_v2.3(fl|+)$ and deleter mice expressing Cre-recombinase constitutively [25]. Thus, exon 2 was deleted by Cre-mediated recombination. $Ca_v2.3(-|-)$ mice were fertile, exhibited no obvious behavioral abnormalities and had the same lifespan as control mice. Parallel breeding of parental inbred mouse lines of $Ca_v2.3$-deficient and control mice ensured their identical background. Further experiments were conducted on $Ca_v3.2(-|-)$ mice in a clean C57Bl/6 background, which were generated as described by Chen et al. [19].

All the mice used in this study were 16 weeks old and were housed at 20–22 °C in Type II Makrolon® cages under a 12 h light-dark cycle (7:00 a.m./p.m.) with unlimited access to food and water. The experiments were conducted in accordance with the European Communities Council Directive for the care and use of laboratory animals and the protocols were approved by the University of Cologne Animal Welfare Committee.

2.2. Mouse handling and langendorff

The isolated perfused Langendorff heart experiments were carried out according to the methods previously established [13]. Briefly, mice were anaesthetized by Ketamin/Xylazine (100/10 mg kg^{-1}) i.p. The isolated hearts were rapidly excised and placed in ice-cold, oxygenated (95% O_2, 5% CO_2) Krebs-Ringer (KR) bicarbonate buffer (pH 7–7.4) (see [13] for composition). Throughout the experiment, the buffer was continuously oxygenated and maintained at 37 °C. The isolated heart was cannulated and retrograde perfusion immediately started, with the flow rate set between 2 to 2.5 ml min^{-1} and controlled with a precision perfusion controlling system (Sarstedt, Nürnberg, Germany).

2.3. Heart treatment

Following a 15-min equilibration phase, the experiment was started using the protocol depicted in Fig. 1. After a baseline period of superfusion with pure KR buffer (2 min), a 20 nM PACAP-27–referred to as PACAP in this paper—(#A9808, SIGMA-ALDRICH, Germany) solution in KR was applied. The system was then switched to the reservoir with a 20 nM PACAP solution supplemented by 50 μM $NiCl_2$ (Merck, Darmstadt-Germany) [9] for suppressing the PACAP-induced effect and washed with pure KR. Except KR for the baseline period, the remaining solutions were applied for 10 min each. After reaching equilibrium, a maximal response was observed within the last minutes of drug superfusion. Specifically, arrhythmia was maximal during this time window following Ni^{2+} application. Therefore, for comparison with baseline conditions, 2 min at the end of each section were selected and evaluated.

2.4. ECG data acquisition and analysis

Spontaneous ECG data were acquired as described previously [13]. Potential difference was recorded at the frequency of 4 kHz with the PowerLab26T. ECG parameters were extracted with the ECG Analysis Module of LabChart Pro v8 for Windows (ADInstruments) and exported for statistical evaluation. Further, during the first 15 min (equilibration phase), hearts displaying arrhythmia for

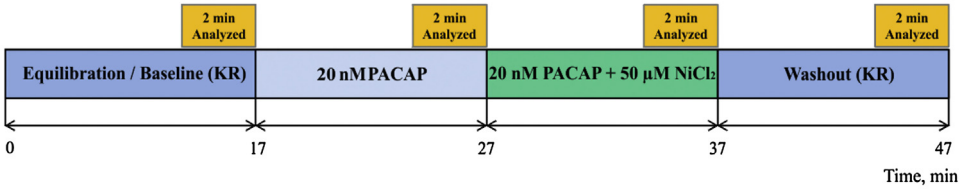

Fig. 1. Schematic representation of the protocol used for treatment of isolated hearts. After excision, the heart was quickly freed from remaining tissues and fat, and cannulated. Following an equilibration phase of 15 min, the first two min were considered as baseline, after which the chemicals were applied for 10 min each as indicated.