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New Drugs for Acute Heart Failure

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Acute heart failure (AHF) is defined as the rapid onset of symptoms and signs of HF secondary to abnormal cardiac function requiring urgent treatment (1). AHF can present itself as acute *de novo* (new onset of AHF in a patient without previously known cardiac dysfunction) or acute decompensation of chronic HF. AHF is associated with high rates of postdischarge mortality and readmission (30-60% within 60 days of admission) and significant healthcare costs (1). In many patients concurrent cardiovascular (coronary heart disease, hypertension, atrial fibrillation, valvular diseases) and noncardiac conditions (diabetes, renal insufficiency, chronic obstructive pulmonary disease, anemia) are often present and may cause or precipitate the AHF (2).

In AHF, the symptoms, clinical course and prognosis are critically dependent on hemodynamics. Thus, the general goals of treatment are: (a) to reduce symptoms (dyspnea and/or fatigue) and signs, (b) to stabilise the hemodynamics (increase cardiac output-CO and decrease pulmonary capillary wedge pressure-PCWP), and (c) to exert favourable effects on outcome, including reductions in the duration of intravenous vasoactive therapy, patient's stay in-hospital, future readmissions and both in-hospital and long-term mortality.

First-line therapy in patients with AHF with congestion and normal or high blood pressure (BP) includes loop diuretics and vasodilators (nitroglycerin, nitroprusside, nesiritide). Although these drugs are effective in reducing fluid overload and acutely relieve symptoms, they may produce serious adverse effects, particularly when used at high dosages (1-3). Inotropic agents (dobutamine, dopamine, phosphodiesterase-3 inhibitors) are indicated in the presence of peripheral hypoperfusion (hypotension, decreased renal function) with or without congestion or pulmonary oedema refractory to diuretics and vasodilators at optimal doses and also as a "pharmacological bridge" to stabilise critically ill patients until definitive life-saving therapy can be undertaken. These agents provide short-term symptomatic and haemodynamic improvement, but their use is associated with increased ventricular ectopy, tachyarrhythmia, ischaemia, increased myocardial oxygen consumption (MVO_2), hypotension and increased post-discharge mortality, particularly in patients with coronary artery disease (4). Thus, early management may influence long-term morbidity and mortality. Because of these well-known limitations, several new drugs are under investigation for the treatment of AHF in an attempt to safely improve haemodynamics and symptoms and possibly long-term survival. The purpose of this review is to analyse the clinical benefit of these new drugs (1-6) (Table 1).

DRUGS FOR AHFS PATIENTS PRESENTING WITH CONGESTION AND NORMAL TO HIGH SBP

These patients present pulmonary and/or systemic congestion and relatively normal CO, and their early man-

Table 1. Investigational pharmacologic agents for the treatment of AHF.

1. **Congestion with normal-to-high BP (diuretics, vasodilator)**
   a. Vasopressin receptor antagonists:
      • V1A receptor - OPC-21268, Relcovaptan
      • V2 receptor - Mozavaptan, Lixivaptan, Atavaptan, Tolvaptan
      • V1A/V2 receptor - Conivactan, JTV-605, CL-385004
   b. A1 adenosine receptor antagonists: BG9719 (CVT-124), BG9928, Rolofillyne (KW-3902)
   c. Endothelin antagonists: bosentan, darusentan, tezosentan
   d. Ularitide (a renal natriuretic peptide)
2. **Normal-to-low systolic blood pressure: levosimendan**
3. **Low BP with or without congestion**
   a. Positive inotropic agents
      • Istaroxime
      • Cardiac myosin activators: CK-1827452, CK-1122534, CK-0689705, CK-121329
      • Metabolic modulators: trimetazidine, ranolazine, etomoxir, ranolazine, L-carnitine

PO-109 OUABAIN-ELICITED APOPTOSIS IN HeLa BUT NOT IN PC12 CELLS

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Objective: We had previously demonstrated that veratridine elicited chromaffin cell death by causing Na^+ and Ca^{2+} overload (Maroto et al. *Eur J Pharmacol* 1994; 270(4): 331-339). Ouabain, a Na^+/K^+ ATPase blocker also causes Na^+ and Ca^{2+} overload of cells. We therefore hypothesised that ouabain might elicit apoptosis through a mechanism implying these actions.
Methods: Excitable PC12 cells (a cell line with characteristics similar to those of chromaffin cells and sympathetic neurons) and non-excitable HeLa cells were used. Apoptosis was measured by flow cytometry and endoplasmic reticulum (ER) and mitochondria (M) [Ca^{2+}] with aequorins targeted to these organelles.
Results: Ouabain 0.1-100 μM (during 24 h) did not elicit apoptosis of PC12 cells; however, the glycoside produced about 50% apoptosis of the HeLa cells within the concentration range 0.1-1 μM; higher concentrations of ouabain did not enhance further apoptotic cell death. A reduction of the [Na^+] to 50 mM and an increase of the [K^+] to 20 mM, and the cell permeable Ca^{2+} chelator BAPTA-AM that should prevent the ionic translocation as a result of Na^+/K^+ ATPase inhibition by ouabain paradoxically increased its apoptotic effects. At concentrations causing apoptosis (0.3 μM) ouabain did not affect the Ca^{2+} fluxes at the ER and M. Neither the mitochondrial Ca^{2+} uniporter blocker KB-R7943 nor the mitochondrial Na^+/Ca^{2+}- exchange blocker CGP37157 or histamine (that releases ER Ca^{2+}) modified the apoptotic effect of ouabain. Free radical scavengers such as vitamin C, trolox and glutation did not affect the apoptotic effects of ouabain either; only N-acetylcistein reduced those effects by 50%.
Conclusion: Our results suggest that apoptosis elicited by ouabain in HeLa cells is unrelated to the widely accepted apoptotic pathway implying cell Ca^{2+} overload and mitochondrial disruption. We suggest two possibilities to explain the ouabain apoptotic effects: (1) ouabain directly activates a cell death receptor; and (2) the binding of ouabain to a Na^+/K^+ ATPase activates an apoptotic cascade unrelated to ion translocation. Because an endogenous ouabain is synthetised in mammals, this apoptotic mechanism may have physiological, physiopathological and pharmacotherapeutic implications.

PO-110 ENDOCANNABINOIDS DIRECTLY BLOCK Kv4.3 and Kv1.5 CARDIAC CHANNELS

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Clinical studies demonstrated that obese patients have an increased risk for atrial fibrillation. Furthermore, in these patients the peripheral endocannabinoid system is upregulated compared to lean patients. Endocannabinoids can be synthesised within the heart and can modulate neuronal and vascular ion channel activity through both receptor-mediated and receptor-independent effects. However, the putative effects of endocannabinoids on human cardiac potassium channels remain unknown. Therefore, the present study was undertaken to determine the effects of the endocannabinoid anandamide (AEA), its metabolically stable analog methanandamide (MEA), 2-arachidonoylglycerol (2-AG), N-palmitoyl ethanolamine (PEA) and lysophosphatidylinositol (LPI) on human cardiac Kv1.5 and Kv4.3 channels, which generate the atrial-specific ultrarapid delayed rectifier current (I_{Kur}) and the transient outward current (I_{to1}), respectively.

Currents were recorded in Ltk- and Chinese hamster ovary cells by using the whole-cell patch-clamp. RT-PCR analysis demonstrated the absence of cannabinoid receptors (CB1, CB2, and GPR55) in both cell types. AEA, MEA, and 2-AG blocked Kv1.5 channels in a concentration-dependent (IC_{50}=1.9 ± 0.1 μM, 2.1 ± 0.1 μM, and 2.6 ± 0.1 μM, respectively) and a voltage-independent manner. PEA and LPI were much less potent for this effect (IC_{50}>50 μM). AEA, MEA, and 2-AG shifted the midpoint of the activation curve to more negative potentials and delayed the deactivation kinetics of tail currents elicited upon repolarisation. AEA, MEA, and 2-AG inhibited Kv4.3 currents in a concentration-dependent manner (IC_{50}=444 ± 34 nM, 693 ± 54 nM, and 215 ± 28 nM, respectively), whereas PEA and LPI were again less potent for this effect. AEA, MEA and 2-AG accelerated the inactivation kinetics and shifted the inactivation curve of Kv4.3 channels to more negative potentials.

AEA, MEA, and 2-AG, but not PEA and LPI, block Kv4.3 and Kv1.5 channels, the K^+ channels that control the height and the duration of the plateau phase of the human atrial action potential. Kv4.3 are more sensitive than Kv1.5 channels, the effects being independent of the interaction with cannabinoid receptors.

PO-111 NITRIC OXIDE INCREASES HUMAN CARDIAC Kir2.1 CHANNELS AND HYPERPOLARISES THE RESTING MEMBRANE POTENTIAL

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Nitric Oxide (NO) hyperpolarises the resting membrane potential (RMP) in atrial preparations obtained from both native and NO-synthase type 3 deficient mice. Kir2.1 channels generate the inward rectifier K^+ current (I_{K1}) that controls the RMP and shapes the final phase of the cardiac action potential (AP) both in human and in mouse myocardium. NO regulates the activity of cardiac Ca^{2+}, Na^+, and many voltage-dependent K^+ channels. However, its putative effects on inward rectifier channels are unknown. Therefore, this work was undertaken to determine the effects of NO on human Kir2.1 channels. Currents were recorded in transiently transfected Chinese hamster ovary cells using the whole-cell and cell-attached patch-clamp. The NO donor S-nitroso-N-acetyl-D,L-penicillamine (SNAP, 200 μM), which released 182 ± 35 nM NO as measured by using a NO-sensitive electrode in the cell chamber, increased both the inward (20.9 ± 5.9% at –120 mV) and the outward (51.7 ± 11.1% at –50 mV) Kir2.1 currents recorded at extracellular ($[K^+]_o$) and intracellular ($[K^+]_i$) K^+ concentrations of 4 and 140 mM, respectively (IC_{50}=75 ± 3 nM NO). Moreover, SNAP rightwardly shifted the reversal potential ($E_{K,C}$=-92.2 ± 2.8 vs $E_{K,SNAP}$=-88.6 ± 3.4 mV, P<0.01). Similar results were obtained when different NO donors (diethylamine NONOate or a NO saturated solution) or $[K^+]_o$ (1, 10 and 140 mM) were used. Single-channel analysis demonstrated that SNAP increased the opening frequency (from 2.6 ± 0.1 to 6.9 ± 0.2 Hz, P<0.05) without modifying the single-channel current amplitude (1.6 vs 1.5 pA, P>0.05), thus leading to an increase in the total open probability (from 0.18 ± 0.01 to 0.26 ± 0.01, P<0.05). SNAP effects were completely prevented by the reducing agent dithiothreitol, suggesting that they could be mediated by the S-nitrosylation of the thiol group of one/various cysteine residues. Indeed, the NO effects were completely abolished in a Kir2.1 channel in which 6 cysteines were mutated (C54V, C76V, C89I, C101L, C149F, and C169V). Point mutations of each of these residues demonstrated that the SNAP effects were only attributable to the S-nitrosylation of Cys76. Our results demonstrated that NO at myocardial physiological concentrations (200-800 nM) regulates the K^+ efflux through Kir2.1 channels, and hence the RMP and the shape of the action potential, via the S-nytrosilation of Cys76.

PO-112 CATECHOLAMINE RELEASE ENHANCEMENT BY AN *EUGENIA PUNICIFOLIA* EXTRACT IN BOVINE ADRENAL CHROMAFIN CELLS

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Background: *Eugenia punicifolia* is a shrub largely distributed in Brazil's Amazonas state, where it is popularly employed in hyperglycaemic states. We previously showed that an aqueous extract of the plant was able to change the pattern of nicotinic antagonist response in rat diaphragm, suggesting the involvement of nicotinic receptors for acetylcholine (ACh) (*J Ethnopharmacology* 2006; 108, 26–30). **Objective:** In the present study we aimed to characterise the effects of dichloromethane extracts of E. *punicifolia* on cholinergic neurotransmission in bovine chromaffin cells.
Methods: Isolated cells were introduced in a microchamber for their superfusion at the rate of 2 ml/min at 37º C with a Krebs-HEPES solution. The liquid flowing from the superfusion chamber reached an electrochemical detector placed just at the outlet of the microchamber that monitors online, under the amperometric mode, the amount of catecholamines secreted. In another series of experiments, extracts were tested for *in vitro* acetylcholinesterase (AChE) and butyrilcholinesterase (BuChE) activities. **Results:** Cells were stimulated with short pulses (5 s) given with a Krebs-HEPES solution containing ACh in the absence or presence of various extract concentrations (0.01–10.000 ng/mL). The lower concentrations of the dichloromethane extracts enhanced the ACh-evoked catecholamine secretion with a maximum effect of 178.0 ± 15.8% at 0.1 ng/mL. In contrast, the higher concentrations reduced catecholamine secretion (51.9 ± 3.90%, at 50.000 ng/mL). None of the concentrations tested inhibited AChE or BuChE enzymes. **Conclusions:** The results obtained in adrenal chromaffin cells corroborate the previously reported action at the rat motor endplate, namely a pro-cholinergic effect of *E. punicifolia*, thus demonstrating that the plant may serve as a new pharmacological tool to assess cholinergic neurotransmission once its main active compounds are identified. The mechanism through which *E. punicifolia* may augment the ACh-evoked catecholamine release is uncertain. We are investigating the possibility that the plant extract could be blocking the Ca^{+2}-activated K+ channels of chromaffin cells.

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