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# Regulation of postburn ischemia by α- and β-adrenoceptor subtypes

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**Abstract**

Deep skin burns are characterised by progressive ischemia secondary to vasoconstriction and thrombosis formation. Burn trauma elicits increased sympathetic activity and elevation of circulating catecholamines acting on adrenoceptors in vascular tissue playing an important role in the regulation of organ blood flow. The present study in rats investigated the role of α- and β-adrenoceptors in the circulatory changes taking place in normal skin and in partial- and full-thickness skin burns using laser Doppler flowmetry. Evaluation was based on intravenous administration of the following adrenergic agonists and antagonists: L-phenylephrine ($\alpha_1$-agonist), prazosin ($\alpha_1$-antagonist), clonidine ($\alpha_2$-agonist), yohimbine ($\alpha_2$-antagonist), prenalterol ($\beta_1$-agonist), terbutaline ($\beta_2$-agonist), and propranolol ($\beta_1$- and $\beta_2$-antagonist). Blood flow in normal skin was reduced by phenylephrine ($p < 0.001$), clonidine ($p < 0.001$) and propranolol ($p < 0.01$), and increased by prazosin ($p < 0.05$), yohimbine ($p < 0.05$), prenalterol ($p < 0.05$) and terbutaline ($p < 0.01$). In partial-thickness burns, blood flow was reduced by phenylephrine ($p < 0.01$), clonidine ($p < 0.01$) and propranolol ($p < 0.05$). In full-thickness burns, only clonidine reduced perfusion ($p < 0.05$). In conclusion, $\beta_1$- and $\beta_2$-adrenoceptors play important role in the physiological regulation of skin perfusion but are of lesser importance for postburn skin perfusion. Vasoconstrictive $\alpha_1$- and $\alpha_2$-adrenoceptors were shown to be tonically active in normal skin and in partial-thickness burns, exerting a negative effect on skin perfusion which was further potentiated by exogenous administration of $\alpha_1$- and $\alpha_2$-agonists and reversed by selective α-blockers. In full-thickness burns, activation of $\alpha_2$-receptors was shown to significantly impair skin circulation, raising a flag of warning for the use of clonidine to treat pain in burn patients.


*Keywords:* Adrenoceptors; Subtype; Burns; Blood flow; Laser Doppler; Skin; Phenylephrine; Clonidine; Yohimbine; Prazosin; Prenalterol; Terbutaline; Propranolol

## 1. Introduction

Studies by the brilliant Danish anatomist, J.B. Winslov (1669–1760) describing the sympathetic nervous system in his textbook of 1752, played a significant role for our understanding of this part of the nervous system and formed a platform for further investigations in the field. The existence of individual adrenoceptors, α and β, was however first proposed by Ahlquist in 1948 [1] based on a series of pharmacological experiments. Further development in the field of biochemical, molecular and genetic techniques has allowed us to identify nine subtypes of adrenoceptors up to date ($\alpha_{1A}$, $\alpha_{1B}$, $\alpha_{1D}$, $\alpha_{2A/D}$, $\alpha_{2B}$, $\alpha_{2C}$, $\beta_1$, $\beta_2$, $\beta_3$), all of which have been shown to be involved in the regulation of vascular tone, either by acting on prejunctional or postjunctional structures [2–6]. The net response to agonists like adrenaline and noradrenaline depends however on the relative

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importance of each adrenoreceptor population in the tissue under investigation and may reveal opposing effects on vascular tone by various receptor subpopulations. The use of transgenic models has allowed us to better understand the role of individual receptors in the regulation of vascular tone ($\alpha_{2A/D}$ in central control of blood pressure, $\alpha_1$ and $\alpha_{2B}$ in peripheral regulation of vascular tone, $\alpha_{2A/D}$ and $\alpha_{2C}$ in the modulation of transmitter release) and their relation to various conditions (e.g. pain) and diseases (e.g. hypertension, Raynaud's disease, Parkinsons disease and several neurological degenerative diseases) [6].

The role of adrenoceptors in the regulation of vascular tone and tissue perfusion is thus well-documented as is the pronounced elevation of circulating catecholamines following burn injury [7] and the crucial role played by progressive ischemia for burn pathophysiology and outcome [8]. In spite, little data is available with regard to the involvement of the sympathetic nervous system and its adrenoceptor subtypes on the dramatic circulatory changes taking place postburn. In the present in vivo study we investigated the importance of $\alpha_1$, $\alpha_2$, $\beta_1$ and $\beta_2$-adrenoceptors on circulatory changes in the normal skin and in the skin following partial- and full-thickness burns.

## 2. Methods

### 2.1. Experiments

Male Sprague–Dawley rats (Møllegaard–Hansen Avelslaboratorie A/S, Denmark) weighing 250–350 g were housed in the animal unit at least 1 week prior to the experiments. In the experimental protocol approved by the Animal Ethics Committee, Göteborg University, the animals were maintained on tap water and standard food pellets (R-34, B&Q Universal, Sollentuna, Sweden) ad libitum, in a 12-h light/darkness cycle at 22 °C.

Anaesthesia was induced with pentobarbital intraperitoneally (60 mg $kg^{-1}$), and maintained with a continuous intravenous infusion of chloralose (1.32 mg $kg^{-1}$ $min^{-1}$) via a catheter (PE-50 polyethylene, Becton-Dickson, USA) in a femoral vein. A catheter in the opposite femoral vein was used for administration of study agents. A tracheal cannula was inserted via a tracheotomy to ensure a free airway. A catheter connected to a pressure transducer (DPT 6000, Berg Medizin teknik, Germany) was inserted into a femoral artery. The transducer was connected to a Grass® polygraph (Model 7, Quincy, MA, USA) allowing for continuous registration of blood pressure and heart rate. Body temperature was kept at 38 °C by placing the animal on a thermo-regulated heating pad connected to a subcutaneous digital thermo-sensor.

### 2.2. Thermal trauma

A burn injury was induced in the closely shaved abdominal skin (Clipper 3M 9604 Remington) using an electrically heated aluminium rod with a 1 $cm^2$ square end surface [9]. The depth of the burn was confirmed in previous studies using a similar burn technique [10]. A thermo-sensor electrode embedded in the bottom surface of the heating rod was connected to a chart writer (Metrawatt SE 120, Asea Brown Boveri, Austria) for temperature recordings. The system was calibrated using a voltage meter by immersing both a reference electrode originating from the thermo-sensor and the bottom plate of the heating probe in 0 °C ice water. The voltage meter registers 0.0 mV and the calibration curve was set to 0 °C. Subsequently, the reference electrode was allowed to remain immersed in 0 °C (ice water) while the heating probe was removed from the water and allowed to equilibrate with room temperature. A new plateau was achieved on the calibration curve and room temperature (21–23 °C) was set as a second fix point on the calibration curve of the chart-writer. Next, the bottom surface of the probe was immersed in water (5 ml) and current to the heating coils of the probe was turned on until the surrounding water reached a boiling point allowing for a third fix point to be set at 100 °C (4.0 mV). At this point (100 °C), current was turned off and the thermo probe was removed from the water, dried and allowed to cool to the desired temperature when it was put in contact with the abdominal skin. In the present experiments, the probe was put in contact with the skin when the temperature reached 55 °C and left in place until the temperature was down to 45 °C, resulting in a full-thickness burn injury in the skin in direct contact with the burn probe (1 $cm^2$), and a partial-thickness burn in the skin surrounding the contact burn (3 mm outside each side of the burn square induced by the heating probe) [10]. This procedure allowed a constant amount of heat energy to be administered to each skin area independent of possible variations in skin temperature and circulation. Probe pressure was standardized to 1.2 N $cm^{-2}$ by pushing down a cylindrical handle compressing in turn a spring on the graded probe.

### 2.3. Laser Doppler measurements

Skin blood flow in the experimental area was measured prior to the burn trauma, and then at 15, 30, 45, 60, 90 and 120 min post-burn. Skin blood flow was measured using laser Doppler flowmetry (Periflux PF3, Perimed, Sweden) with a standard flow probe (PF 308, Perimed, Sweden). Three sets of measurements were performed. In one set, blood flow was monitored within the boundaries of the skin area in direct contact with the burn probe (1 $cm^2$) representing a full thickness burn [19]. Five different measurements (centre and corners) were performed at each time interval and the mean value was used for calculations. In a second set, four laser Doppler measurements were performed in the skin surrounding the contact burn (3 mm outside each side) and the mean value was given. The latter measurements represented a partial-thickness burn [19]. In the third set, blood flow was