Life Sciences 83 (2008) 85–91

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Role of VR1 and CB1 receptors in modelling of cardio-respiratory response to arvanil, an endocannabinoid and vanilloid hybrid, in rats

Beata Kopczyńska *

Laboratory of Respiratory Reflexes, PAS Medical Research Center, 5 Pawińskiego St. 02-106 Warsaw, Poland

ARTICLE INFO

Article history:
Received 12 November 2007
Accepted 4 April 2008

Keywords:
VR1 receptor
CB1 receptor
Arvanil
Cannabinoids
Regulation of breathing
Regulation of blood pressure
Rat

ABSTRACT

Cardio-respiratory effects of an intravenous injection of arvanil, a structural "hybrid" between capsaicin and anandamide, were investigated in 40 urethane-chloralose anaesthetized and spontaneously breathing rats. In the group of rats the response to arvanil was checked to establish the appropriate dose of the drug. To analyze the pattern of the cardio-respiratory effects rats were challenged with bolus injection of arvanil (0.8 mg kg^{-1}) into the femoral vein. Administration of the drug evoked, in all tested rats, a significant increase of tidal volume (V_T) and diaphragm activity, hypertension coupled with a fall in respiratory rate (f). To test the contribution of vanilloid (VR1) and cannabinoid (CB1) receptors to post-arvanil response, administrations of the drug were preceded by nonselective VR1 antagonist ruthenium red, selective VR1 antagonist SB366791 or selective CB1 antagonist AM281. All antagonists eliminated an increase in V_T but failed to block the hypertension evoked by arvanil. Ruthenium red as well as SB366791 abolished post-arvanil fall in respiratory rate. The rise of diaphragm activity was totally eliminated by ruthenium red and markedly reduced by SB366791. AM281 blockade of post-arvanil changes in f and diaphragm activity was ineffective. These findings indicated that the post-arvanil rise of V_T was mediated by both VR1 and CB1 receptors. Only vanilloid receptors were involved in the increase of diaphragm activity and decrease of respiratory frequency. Hypertensive response to arvanil might depend on different types of receptors.

Introduction

Arvanil ($\Delta^{5,8,11,14}$-*cis* eicosatetraenoyl-*N*-acyl-vanillyl-amide), metabolically stable, non-pungent hybrid between capsaicin and anandamide synthesized by Melck et al. (1999) activates both cannabinoid CB1 (but not CB2) and vanilloid receptors. Arvanil also acts as a potent inhibitor of the anandamide transporter (Melck et al., 1999) and seems to be capable of activating CB1 receptor indirectly by enhancing levels of anandamide. That may be the reason why arvanil, having similar affinity for the cannabinoid CB1 receptors to anandamide (Di Marzo et al., 2000a, 2001b), is functionally a more potent activator of CB1 receptor than anandamide itself (Di Marzo et al., 2002a). Some studies call into question the existence of "anandamide transporter" and suggest that arvanil is, in fact, an inhibitor of fatty acid amide hydrolase (FAAH) which regulates the uptake of endogenous anandamide. It was proved that arvanil is a more potent stimulator of the vanilloid receptors than capsaicin (De Petrocellis et al., 2000; Ross et al., 2001). Arvanil administered systemically was found to be extremely potent in the mouse "tetrad" of cannabinoid effects like hypothermia, analgesia, catalepsy and in inhibiting spontaneous activity (Di Marzo et al., 2000a; Lo et al., 2003).

The intravenous capsaicin challenge usually evoked hypotension and hyperventilation occasionally preceded with 5–10 s lasting apnoea (Smith and McQueen, 2001). Post-capsaicin respiratory and cardiovascular response is mediated by vanilloid receptors (VR1) (Smith and McQueen, 2001). Intravenous anandamide administration in a dose of 1 mg kg^{-1} always caused apnoea (Kopczyńska and Szereda-Przestaszewska, 2006; Kopczyńska, 2007), bradycardia (Lin and Lee, 2002) and hypotension (Varga et al., 1995; Lake et al., 1997; Malinowska et al., 2001; Kopczyńska, 2007). Apnoea was followed by the fall in tidal volume (Kopczyńska and Szereda-Przestaszewska, 2006). Apnoeic and hypotensive response to anandamide was mediated by both vanilloid VR1 and cannabinoid CB1 receptors. Post-anandamide decline of tidal volume (V_T) might depend on different type of receptors (Kopczyńska, 2007).

The mechanism of arvanil cardio-respiratory effects is worth exploring on account of its ability to alleviate hyperkinesia typical of rat model of Huntington's disease (Di Marzo et al., 2001a; De Lago et al., 2005), spasticity, pain (Brooks et al., 2002), tremor and other signs of disease in rat model of multiple sclerosis (Cabranes et al., 2005) antitumor (De Lago et al., 2006) and anti-inflammatory action (Di Marzo et al., 2000b, 2001b) and possible usage of cannabinoids and their derivatives as drugs.

To improve the characteristic of arvanil as a hybrid agonist of VR1/CB1, present experiments were designed to define: 1) the breathing pattern and cardiovascular effects induced by this drug and dose-dependent progress of cardio-respiratory response; 2) the contribution

* Tel.: +48 22 6086522; fax: +48 22 6685532.
E-mail address: kopczynb@cmdik.pan.pl.

0024-3205/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2008.04.023

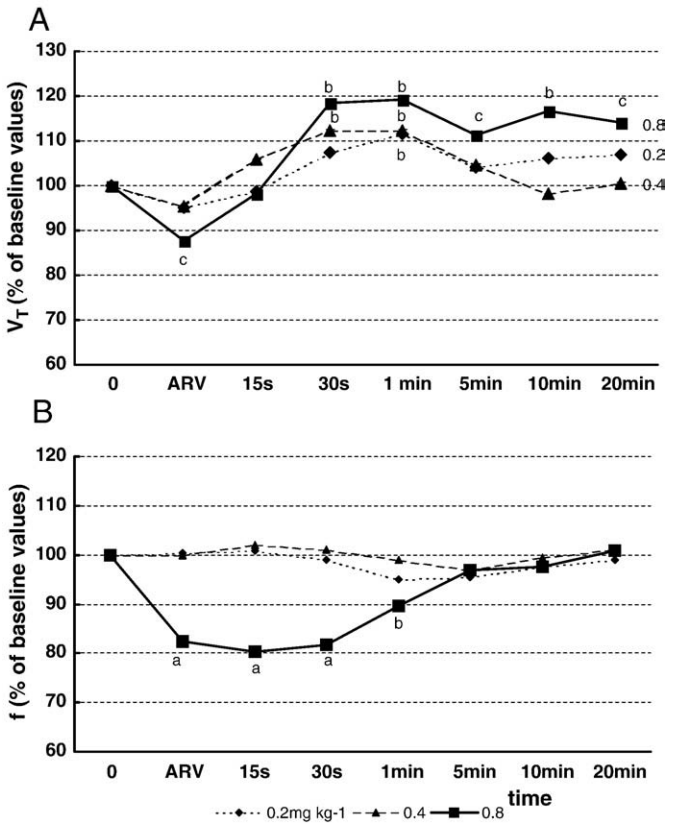

Fig. 1. Dose related effect on tidal volume (V_T) (A) and respiratory rate (f) (B) evoked by arvanil (0.2–0.8 mg kg^{-1}) in neurally intact rats. Note the most apparent response to the dose of 0.8 mg kg^{-1}. $^{a}p<0.001$, $^{b}p<0.01$, $^{c}p<0.05$ vs. baseline (pre-arvanil values), Duncan's test; $n=13$.

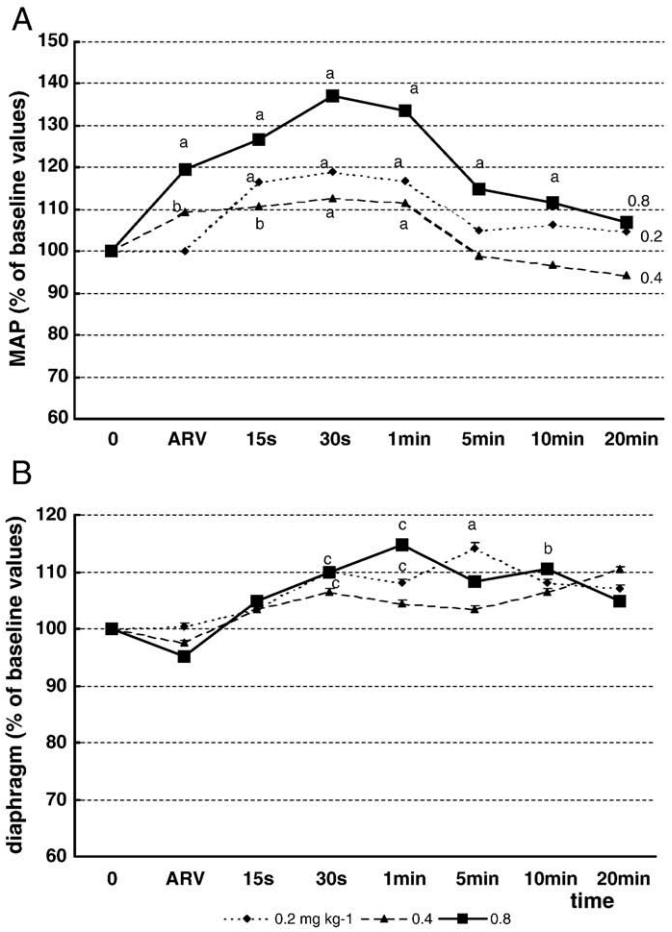

Fig. 2. Dose-dependent response on mean arterial pressure (MAP) (A) and diaphragm activity (B) evoked by arvanil (0.2–0.8 mg kg^{-1}) in neurally intact rats. Note the most apparent response to the dose of 0.8 mg kg^{-1}. $^{a}p<0.001$, $^{b}p<0.01$, $^{c}p<0.05$ vs. pre-arvanil values, Duncan's test; $n=13$.

of VR1 and CB1 receptors in the post-arvanil cardio-respiratory response.

Methods

Forty adult male Wistar rats (195–250 g body weight) were anaesthetized with an intraperitoneal (i.p.) injection of urethane (600 mg kg^{-1}) and α-chloralose (120 mg kg^{-1}). Additional doses of urethane and α-chloralose (Fluka AG) were administered intravenously (i.v.) to maintain possibly constant level of the surgical anesthesia. Rats were placed supine and spontaneously breathed room air. An incision was made in the trachea below the larynx, and the cannula inserted into the caudal end was connected to a pneumotachograph. Femoral vein and artery were catheterized for administration of supplemental anesthesia and drugs and to monitor blood pressure, respectively.

Ethical approval for the experimental procedures used in this study was obtained from the local animal care committee. All animal procedures were conducted in accordance with NIH Guide for the Care and Use of Laboratory Animals.

Arterial pressure was measured with BP-2 pressure monitor (Columbus Instruments) and mean arterial pressure (MAP) was calculated. Volume signals were recorded from a pneumotachograph (RSS 100HR Research Pneumotach System). Electromyogram of the costal diaphragm was recorded with bipolar electrodes, amplified with NL 104 amplifier (Digitimer) filtered and measured with a model AS 101 (Asbit) leaky integrator (time constant=100 ms). All recordings were registered with Omnilight 8 M 36 apparatus (Honeywell). Rectal

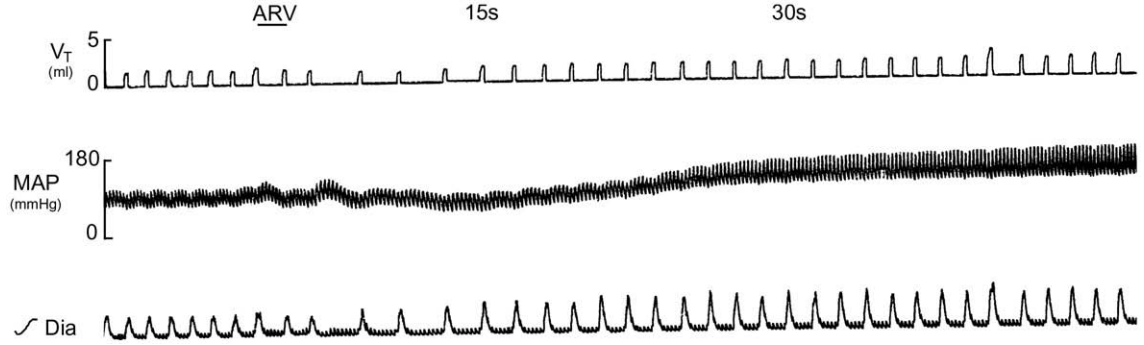

Fig. 3. Cardio-respiratory response to intravenous injection of arvanil (dose of 0.8 mg kg^{-1}) in the rat. Arvanil injection marked by a dash above the upper record. The rise in tidal volume, diaphragm activity and mean arterial pressure coupled with decrease in respiratory frequency. V_T — tidal volume; MAP — mean arterial pressure; Dia — integrated myogram of the diaphragm.