ELSEVIER

Autonomic Neuroscience: Basic and Clinical 125 (2006) 16 – 21

AUTONOMIC
NEUROSCIENCE:
Basic & Clinical

www.elsevier.com/locate/autneu

Visceral-related area in the rat insular cortex

V. Bagaev [✠], V. Aleksandrov [*]

Pavlov Institute of Physiology, nab.Makarova 6, Sankt-Petersburg 199034, Russia

Received 14 January 2006; received in revised form 14 January 2006; accepted 14 January 2006

Abstract

The insular cortex interacts with the bulbar autonomic nuclei providing autonomic manifestations accompanying several neurological and psychosomatic disorders. The aim of our study was to identify the sites within the insular cortex, which could be responsible for the gastrointestinal, respiratory and cardiovascular responses. The main methods used were microinjections of HRP into several parts of the bulbar dorsal vagal complex and microstimulation of the insular cortex. It has been found that a compact group of neurons located in the middle level of the rat insular cortex projects directly to the specific "gastric" part of the dorsal vagal complex. Retrograde labelled cell bodies were revealed in the V layer of the disgranular and agranular insular cortex. Microstimulation of the sites within the middle level of the rat insular cortex produced gastric motor responses and a decrease in inspiratory airflow without significant alteration in respiratory cycle duration. More caudal microstimulation produced an increase in respiratory airflow and decreased respiratory cycle duration. These responses were usually accompanied by changes in the level of blood pressure. It is concluded that autonomic representation in the rat insular cortex is organised in a viscerotopic manner. The inhibitory respiratory zone overlaps with the gastrointestinal control area in the middle part of the insular cortex. More caudally, the excitatory respiratory zone overlaps with the cardiovascular area. On the basis of these experimental results and the data of others authors the general scheme of autonomic representation in the rat insular cortex is discussed.

Keywords: Autonomic control; Insular cortex; Dorsal vagal complex; Visceral functions

---

1. Introduction

I. Pavlov and his followers revealed the fundamental facts regarding the role of cerebral cortex in the autonomic control. Following the phenomenon of "psychic" secretion of gastric juice (Pavlov, 1902), it has been discovered that autonomic activity can be modified by classic (Pavlovian) conditioning and, moreover, visceral afferent impulses can provide a conditioning stimulus in the procedure of classic conditioning (Bykov, 1947). The concept of cortico-visceral relationships, which was developed by Pavlov's school, has formed the basis for the theory of cortico-visceral pathology (Bykov and Kurtsin, 1954). The idea of cortico-visceral reflex arc is an important element of this theory (Chernigovsky, 1967). Facts and ideas of cortico-visceral relationships as well as Thorndike's methodology exerted influence on the theory and practice of instrumental training of visceral systems (Miller, 1969). This technique considers a cerebral cortex as an essential element in the system of autonomic control.

Unfortunately several essential questions concerning the role of certain cortical areas in autonomic control and neurophysiological mechanisms of cortical influences on visceral functions are stilled unanswered. Even the existence of specific zones of visceral cortex playing a key role in the autonomic control was under question sometimes. Rapid progress in this area occurred after the introduction of new experimental methods. Neuronal markers were used for studying connections within the nervous system and microstimulation of the cortex was introduced into the physiological experiments. It had been found that three cortical

---

[*] Corresponding author. Present address: Department of Human and Animal Anatomy and Physiology, Herzen State Pedagogical University, Moika Emb. 48., Sankt-Petersburg, 191186, Russia. Tel.: +7 812 3116088; fax: +7 812 3121195.

E-mail address: n_aleks@yahoo.com (V. Aleksandrov).

[✠] The editors regret to announce that Professor Bagaev recently died following heart surgery.

1566-0702/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.autneu.2006.01.006

areas play major roles in autonomic control. These are infralimbic (medial prefrontal) cortex, insular (lateral prefrontal) cortex and somatic sensory and motor cortices (Cechetto and Saper, 1990; Saper, 2002). According to a theory most popular at present, the infralimbic cortex is a visceromotor cortex, whereas the insular cortex is a viscerosensory cortex (Hurley-Gius and Neafsey, 1986; Öngür and Price, 2000; Saper, 2002).

There is evidence that visceral sensory information reaches the insular cortex (Cechetto and Saper, 1990). However, there is a pool of experimental data concerning insular cortex which does not correspond with general scheme described above. Firstly, electrical stimulation of insular cortex produces variety of autonomic effects. It changes for example blood pressure level, heart and respiratory rates, gastric and small bowel motility (Kaada, 1951; Hoffmann and Rasmussen, 1953; Penfield and Faulk, 1955; von Euler and Folkow, 1958; Delgado, 1960; Hoff et al., 1963; Hall et al., 1977; Ruggiero et al., 1987 etc.). Secondly, major efferent projections have been identified from the insular cortex to the central nucleus of amygdala, lateral hypothalamic area, parabrachial nucleus, vago-solitary complex, and others autonomic centres (Ross et al., 1981; Saper, 1982; Shipley, 1982; van der Kooy et al., 1982, 1984; Yasui et al., 1985, 1991; Ruggiero et al., 1987). Moreover, it has been found that certain efferent projections are responsible for the autonomic effects of insular stimulation (Cechetto and Chen, 1990). Therefore we conclude the evidence that the insular cortex acts as a visceral sensorimotor cortex is good strong.

The main aim of our study was to describe a visceral motor area within the rat insular cortex and to compare it with the visceral sensory representation.

2. Materials and methods

All animal procedures were conducted in accordance with the ethical guidelines of the European Community Council Directives 86/609/EEC. The experiments were performed on adult male Wistar rats in the weight range 230–270 g after fasting for 16 h with free access to water.

First of all experiments were performed to find insular neurones sending efferent projections to “gastric” neurones within dorsal vagal complex (DVC) and modulating their activity. DVC consists of the nucleus of the solitary tract (NTS) and the dorsal motor nucleus of the vagus nerve. These nuclei participate in gastric motor vago-vagal reflex activity (McCann and Rogers, 1992; Bagaev and Panteleev, 1994). Microinjections of retrograde transported tracer horseradish peroxidase (HRP) were made into the DVC. Anterograde transport of wheat germ agglutinin conjugated with HRP (WGA-HRP) was studied after microinjections of this tracer into the insular cortex.

Animals were anaesthetised with Nembutal (40 $\text{mg}\cdot\text{kg}^{-1}$, i.p.) and fixed in a stereotaxic unit. An occipital bone and caudal part of the cerebellum were removed. In some experiments ($n=8$) DVC was injected with 50–100 nl 20% solution of HRP (Sigma, Type VI, RZ=3.0) through the glass micropipette (tip diameter of about 15 μm). In other experiments ($n=3$) 25–50 nl of 1% solution of WGA-HRP (Sigma) were injected into the insular cortex. In 72 h after injection the animals were anaesthetised again (urethane, 1400 mg/kg, i.p.) and perfused intracardially as described previously (Aleksandrov et al., 1996). The brain was removed and cut in 50 μm frontal sections with subsequent processing by the diaminobenzidine method (Mesulam, 1982). Sections were made up with 0.1% safranine solution and used for identification of the HRP labelled neurones in the insular cortex. The locations of labelled cell bodies, fibres and terminals were determined by atlas (Paxinos and Watson, 1982) and marked on line drawings.

Next, parts of the present study were devoted to examination of the autonomic effects produced by microstimulation of the insular cortex. Gastric effects produced by areas containing the neurones sending axons to the DVC were of particular interest at first stage. Then gastric, cardiovascular, and respiratory effects were identified in various parts of rat insular cortex and locations of responsive sites were mapped.

The animals ($n=26$) in these stimulation experiments were anaesthetised with urethane (i.p. 1400 mg/kg) to a level which was sufficient to eliminate noxious somatic reflexes. The depth of anaesthesia was monitored by responses to tactile stimuli and corneal reflex. Rectal temperature was maintained at 37 ± 0.2 °C. The animals were intubated with an endotracheal tube. The femoral artery was cannulated for the measurement of arterial blood pressure using pressure transducer. The femoral vein was cannulated for administration of drugs. Indexes of breathing pattern were found from airflow measured by two-sidearm cannula designed for respiratory airflow measurements in small animals (Mortola and Noworaj, 1983). One side of the cannula was attached to the endotracheal tube another was free. Both sidearm of cannula were connected to a differential pressure transducer coupled with an amplifier for airflow recording. Airflow was integrated to tidal volume. Minute ventilation was calculated from the mean tidal volume and respiratory frequency of ten consecutive respiratory cycles. Gastric motility was monitored by the endogastric insertion of a latex balloon. The balloon were filled with 1.0 ml of air and connected via a catheter (i.d., 0.5 mm; length, 20 cm) to a differential pressure transducer. Special experiments were done to study insular influences upon vagal induced gastric motor reflex ($n=6$). In order to evoke this reflex, the left cervical vagal nerve trunk of anaesthetised rats was cut and the central end was stimulated with series of rectangular pulses of 0.5 ms duration, frequency of 10–20 Hz and 200–500 μA current strength.