

## Short communication

## Pentylentetrazole-induced parasympathetic blood flow increase in the lower lip of the cat

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**Abstract**

The pentylentetrazole (30 mg/kg i.v.)-induced blood flow increase in cat lip was more marked on the sympathectomized side than on the intact side ( $P < 0.01$ ). This difference is probably dependent on the degree of simultaneous activation of the sympathetic nerve elicited by pentylentetrazole administration. The blood flow increases were markedly suppressed by prior treatment with hexamethonium (10 mg/kg i.v.), an autonomic ganglion blocker ( $P < 0.01$ ). Combined section of the facial and glossopharyngeal nerve roots completely abolished the blood flow increases elicited by pentylentetrazole administration ( $P < 0.01$ ), but section of either the facial or glossopharyngeal nerve root alone failed to produce complete abolition ( $P < 0.05$ ). These results indicate that the relevant parasympathetic vasodilator fibers originate not only from the glossopharyngeal, but also the facial nerves and that both participate in pentylentetrazole-induced vasodilatation in the cat lower lip.

**Keywords:** Pentylentetrazole; Parasympathetic reflex; Vasodilatation

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**1. Introduction**

Intense activation of the sympathetic nerves has been reported to occur as a result of experimentally induced seizure (Faraci et al., 1986; Mueller et al., 1979). Extracranial parasympathetic vasodilator fibers also seem to be stimulated by seizure (Faraci et al., 1986), but their effects tend to be masked by the vasoconstrictor response elicited by the simultaneous activation of the sympathetic supply. This indicates that seizure-inducing drugs such as pentylentetrazole and bicuculline directly stimulate neurons of the brain-stem involved in central regulation of the autonomic nervous system. We have previously suggested that there is a dual innervation of the cat lower lip by two groups of parasympathetic vasodilator fibers originating from the facial and glossopharyngeal nerves (Izumi and Karita, 1991, 1993a). Despite this dual innervation, the only parasympathetic vasodilator fibers involved in trigeminally or viscerally induced reflex vasodilatation

in the lower lips of cats are those running in the glossopharyngeal nerve (Izumi and Karita, 1992, 1993b,c), indicating that the participation of the facial nerve-mediated vasodilator system in the cat lower lip is less well-defined or as yet unrecognized.

The aims of this study were: firstly, to investigate whether centrally mediated activation of the autonomic nervous system caused by pentylentetrazole administration can elicit a vasodilator effect in the lower lip of sympathectomized cats; secondly, if such a response occurs, to examine any difference in its magnitude on the intact and sympathectomized sides, and lastly to establish whether the parasympathetic vasodilator fibers running in the facial nerve are involved.

**2. Materials and methods**

Experiments were performed on 16 cats of either sex weighing 1.0–4.0 kg. The cats were initially anaesthetized with ketamine hydrochloride (30 mg/kg i.m.) and then with a mixture of chloralose (50 mg/kg i.v.) and urethane (100 mg/kg i.v.). One cephalic vein was cannulated to allow drug injection and one femoral

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artery was cannulated and connected to a Statham pressure transducer to monitor systemic arterial blood pressure. The animals were intubated, paralyzed with pancuronium bromide (Mioblock, Organon; 0.4 mg/kg initially followed by a constant infusion at a rate of approximately 0.2 mg/kg/h) and artificial ventilation was maintained with a Harvard ventilator. The sympathetic trunk was always sectioned unilaterally more than 1 h before the experiment. Ten minutes before injection of pentylene-tetrazole (30 mg/kg i.v.), an additional injection of pancuronium bromide (0.2 mg/kg i.v.) was given to ensure complete skeletal muscle paralysis. Rectal temperature was maintained at 37–38°C with a heating pad.

When required, the root of the facial and/or glossopharyngeal cranial nerves was divided from its origin in the brainstem after extensive craniectomy. To examine the effects of hexamethonium (autonomic ganglion blocking agent, 10 mg/kg i.v.) on the pentylene-tetrazole-induced blood flow increase, hexamethonium was administered 10–15 min prior to a further administration of pentylene-tetrazole. Blood flow changes in the lower lip were monitored using a laser Doppler flowmeter (Canon LC-1, Tokyo, Japan, Moor Instruments MBF3D, Devon, UK or Advance ALF21R, Tokyo, Japan) as described previously (Izumi and Karita, 1992, 1993a,b,c). At the end of the experiment, the cat was killed with an overdose (about 150 mg) of Nembutal.

The changes in blood flow were expressed as the ratio of the peak blood flow during the vasodilatation elicited by a seizure to the highest level of the fluctuation in baseline blood flow before pentylene-tetrazole administration (inset in Fig. 1) and as the ratio of post-treatment response to pre-treatment response, expressed as a percentage (Fig. 2). All numerical data are given as the means  $\pm$  S.E. The significance of blood flow changes was tested using a paired Student's *t*-test (inset in Fig. 1 and Fig. 2 (facial nerve section)) and the Welch analysis (Fig. 2, glossopharyngeal nerve section and hexamethonium). Differences were considered significant at a level of  $P < 0.05$ .

### 3. Results

A typical example of the blood flow increase elicited by an i.v. injection of pentylene-tetrazole (30 mg/kg) in the intact (right) and sympathectomized (left) sides of the cat lower lip is shown in Fig. 1 and the averaged data for the ratio of the peak blood flow level elicited by a seizure to the highest level of the fluctuation in baseline blood flow before pentylene-tetrazole administration are shown in the inset in Fig. 1. The pentylene-tetrazole-induced blood flow increase in cat lower lip was more marked on the sympathectomized

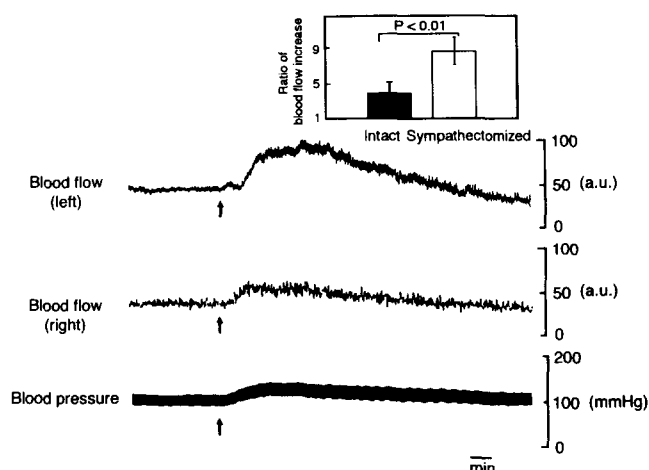


Fig. 1. Typical examples of effects of pentylene-tetrazole on lip blood flow on the intact (right) and sympathectomized (left) sides and on arterial blood pressure. Pentylene-tetrazole (30 mg/kg i.v.) was injected where indicated by arrows. Abscissa: time (min). Ordinate: blood flow in the lower lip on left and right sides and arterial blood pressure shown in arbitrary units (a.u.) and mm Hg, respectively. Inset compares the blood flow increases in response to pentylene-tetrazole administration (30 mg/kg i.v.) on the intact (filled column) and sympathectomized sides (open column), calculated from the data from 12 cats (means  $\pm$  S.E.). *P* value refers to comparison between the two. The number of animals used was 12.

side than that on the intact side ( $P < 0.01$ ,  $n = 12$ ). There was also a prolonged increase in mean arterial blood pressure (from  $104.7 \pm 7.8$  to  $124.3 \pm 3.8$  mm Hg,  $P < 0.05$ ,  $n = 5$ ). The blood flow increases were strongly suppressed ( $P < 0.05$ ,  $n = 4$ ) by prior treatment with hexamethonium (10 mg/kg i.v.), an autonomic ganglion blocker. Combined section of the facial and glossopharyngeal nerve roots completely abolished the blood flow increases elicited by pentylene-tetrazole (30 mg/kg) ( $P < 0.001$  vs. before sectioning the nerves) but section of either the facial ( $P < 0.05$  vs. before facial nerve section) or glossopharyngeal nerve root alone

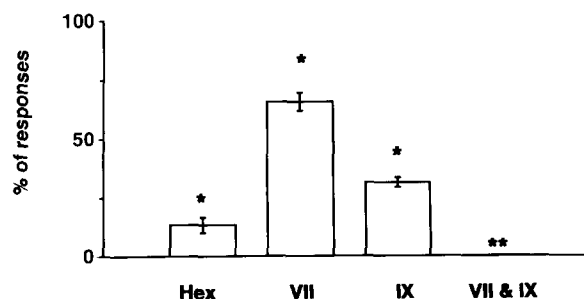


Fig. 2. Effects of hexamethonium (Hex, 10 mg/kg i.v.) and section of the facial (VII) and/or glossopharyngeal (IX) nerves on the blood flow increase elicited in cat lower lip by i.v. administration of pentylene-tetrazole (30 mg/kg). The magnitude of the blood flow responses after each maneuver is expressed as a percentage of the pre-treatment response and is given as mean  $\pm$  S.E. Statistical significance was calculated using a paired *t*-test. \*  $P < 0.05$ , \*\*  $P < 0.01$  vs. before treatment. The number of animals used was 4.

( $P < 0.05$  vs. before facial nerve section) failed to produce complete abolition (Fig. 2). Section of the glossopharyngeal was more effective than section of the facial nerve ( $P < 0.05$ ). During these experiments, both the blood flow increase on the contralateral intact side and the systemic blood pressure increase induced by pentylene-tetrazole administration were the same as the control response (before any nerve section) regardless of whether the facial or glossopharyngeal nerve was unilaterally sectioned alone or the two were sectioned together (data not shown).

#### 4. Discussion

Parasympathetic vasodilator responses elicited by seizure-inducing drugs such as pentylene-tetrazole and bicuculline are usually not consistent between organs or tissues even in animals whose cervical sympathetic trunk has been cut: thus, seizure markedly increased blood flow in the tongue, but not in the submandibular gland despite the fact that both vasodilator responses are mediated by parasympathetic fibers in the chorda tympani (Faraci et al., 1986). Lip blood flow was measured to investigate the effects of pentylene-tetrazole-induced blood flow changes in the present studies for the following reasons. According to histological and physiological studies on the lower lip of cats (Gibbins et al., 1984; Izumi and Karita, 1992, 1993a; Kaji et al., 1988; Kuchiiwa et al., 1992; Karita and Izumi, 1994), lip blood vessels are richly innervated by parasympathetic as well as sympathetic nerve endings and can be dilated reflexly in response to somatosensory stimulation via activation of the parasympathetic pathway. In addition, it has been found that lip blood flow is less responsive to changes in systemic arterial blood pressure than that of the tongue and submandibular gland, presumably because lip blood vessels have fewer anastomoses.

The present study has demonstrated a pentylene-tetrazole-induced blood flow increase in the cat lower lip that is more profound on the sympathectomized side than on the intact side (Figs. 1 and 2). In 8 out of 12 stimulations, such a lip blood flow increase was observed on pentylene-tetrazole administration, while in 4 stimulations there was no change on the intact side, suggesting that the magnitude of the blood flow increase in response to pentylene-tetrazole administration may be dependent on the degree of simultaneous activation of sympathetic vasoconstrictor fibers. Faraci et al. (1986) have previously reported that i.v. administration of bicuculline does not elicit a blood flow increase in the tongue or submandibular gland in intact rats. This may be related to the degree of sympathetic activation or it may be due to the different experimen-

tal conditions in the two studies. We compared the effects of i.v. administration of pentylene-tetrazole on blood flow on the left and right sides of the same individual animal (cat) using a laser Doppler flowmeter, while Faraci et al. (1986) compared responses in intact animals with those in bilaterally sympathectomized animals (rats) using a microsphere method. The tissue studies were also different, as were the anesthetics used.

The lip blood flow increase elicited by pentylene-tetrazole administration in our study was markedly reduced by prior treatment with hexamethonium, an autonomic ganglion blocker. This suggests that the blood flow increase induced in cat lower lip by pentylene-tetrazole administration is due to activation of parasympathetic pathways as the sympathetic supply was cut. The lip blood flow increase in response to pentylene-tetrazole was found to be completely suppressed by section of the root of the glossopharyngeal and that of the facial nerve together but not by that of the glossopharyngeal nerve root alone (Fig. 2), indicating that parasympathetic vasodilator fibers originating from the facial nerve participate in the pentylene-tetrazole-induced vasodilatation in the cat lower lip. In this important respect, the response to the seizure-inducing drug differs from the somato-parasympathetic reflex response reported previously in cat lower lip (Izumi and Karita, 1992, 1993b,c) in which only the glossopharyngeal vasodilator system is involved. This suggests a different central pattern of stimulation of the parasympathetic nerves in pentylene-tetrazole administration and in somatic stimulation.

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