〔Original〕

Anterior hypothalamic inhibition of reflex parasympathetic vasodilatation in the lower lip and palate of anaesthetized cats

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Abstract

1. The aim of the present study was to test for modulation by the hypothalamus of the parasympathetically mediated reflex vasodilatation in lower lip and palate evoked by electrical stimulation of the central cut end of the lingual nerve (LN) in anaesthetized vago–sympathectomized cats.
2. Electrical stimulation of the anterior hypothalamus consistently elicited an intensity (50–500 μA) –dependent attenuation of the lip blood flow increase reflexly evoked by LN stimulation, at intensities that did not elicit a pressor effect. The optimum stimulus frequency for the inhibitory effect was 50 Hz.
3. The greatest inhibitory effect was evoked from the periventricular region of the anterior hypothalamic area.
4. Prior administration of a relatively specific antagonist of γ–aminobutyric acid type A ($GABA_A$) receptors,picrotoxin, at a dose of 1 mg/kg (i.v.) completely abolished the inhibitory effect of anterior hypothalamic stimulation at any of the stimulus intensities and frequencies used, suggesting that the inhibitory effect of hypothalamic stimulation might be exerted via a GABA–like effect.
5. Microinjection of D, L–homocysteic acid (1 M, 0.2 μl), an excitatory amino acid, into the anterior hypothalamus significantly inhibited the lip blood flow increase elicited reflexly by LN stimulation, suggesting that cell bodies in the anterior hypothalamus are responsible, at least in part, for the inhibitory action.
6. This is the first demonstration of a modulation by the anterior hypothalamus of a non–vagal parasympathetic reflex mechanism involving the oro–facial area of the cat.

Key words：Anterior hypothalamic inhibition, Parasympathetic, Vasodilatation, Cat

Introduction

There have been many investigations of the physiological role of the hypothalamus in anaesthetized and conscious animals. These studies can broadly be classified into two groups: those that examined the role of the hypothalamus in emotional behaviour and the related autonomic changes, such as those involving the cardiovascular system (see review by Jordan 1990; Kojima et al., 1995), and those that examined the modulation by hypothalamic neurons of somato (or visceral)–autonomic reflex responses such as the baroreceptor reflex (see review by Spyer, 1990). All of these investigated only the influence of hypothalamic stimulation over either the sympathetic system or the vagal portion of the parasympathetic system (or both). To our knowledge, no study has been made of a possible hypothalamic influence over reflex parasympathetic effects that are not mediated via the vagus.

It has generally been considered that the defence–alerting reaction, which involves a rise in arterial blood pressure, is fully integrated at the hypothalamic level, the response to appropriate hypothalamic stimulation incorporating both autonomic and behavioural components (e.g. Abrahams et al., 1960, 1964; Yardley & Hilton, 1986). However, despite much effort in the past, the precise physiological role of the hypothalamic nuclei in this response remains unclear, since,

Received date : 2011, 9, 30

interestingly, microinjection of an excitatory amino acid does not necessarily elicit a cardiovascular or behavioural response similar to that evoked by electrical stimulation of the same hypothalamic area (Bandler, 1982; Hilton & Redfern, 1986).

We have previously reported that parasympathetic vasodilator fibers run in the glossopharygeal and facial nerves to supply the lower lip, palate and masseter muscle of the cat and rat (presumably originating from the inferior and superior salivatory nuclei, respectively), and that trigeminal spinal nucleus is an important bulbar relay for lingual nerve–evoked parasympathetic reflex vasodilatation, and we suggested a possible physiological role for these vasodilator fibers in somato–parasympathetic reflex vasodilatation (Izumi & Karita, 1991 , 1992, 1993; Mizuta et al., 2002; Ishii et al., 2005, 2007, 2009, 2011; Sakurai et al., 2006; Koeda et al., 2009). The orofacial area of the cat and rat receives a rich parasympathetic supply, judging from the distribution of fibers containing VIP (vasoactive intestinal polypeptide) –like immunoreactivity (Gibbins, Brayden & Bevan, 1984, Niioka et al., 2009), and the vasomotor control of this area seems to be predominantly regulated by parasympathetic, rather than sympathetic, reflex mechanisms (Izumi & Karita, 1992). Thus, measurement of vasomotor changes in this area was considered by us an appropriate tool for an investigation of the possible modulation by the hypothalamus of non–vagal parasympathetically mediated reflex mechanisms. To this end, the effects of electrical and chemical stimulation within the hypothalamus were investigated on the parasympathetically mediated reflex vasodilatation in the lower lip and palate.

The experiments were performed on anaesthetized cats in which cardiovascular effects elicited by hypothalamic stimulation were minimized by cutting the vagus nerve in the neck bilaterally and by delivering the stimulus at intensities less than that needed to raise arterial blood pressure. Furthermore, the sympathetic trunk in the neck was sectioned bilaterally prior to any stimulation so as to eliminate any activation of sympathetic nerves to the oro–facial areas by hypothalamic or reflex stimulation. From the results, we conclude (i) that an attenuation of the parasympathetically mediated reflex vasodilatation in the lower lip and palate of vago –sympathectomized cats can be evoked by electrical stimulation of anterior hypothalamus underlies at levels that cause no blood pressure increase and (ii) that GABA may be the inhibitory transmitter mediating this effect at synapses somewhere in the parasympathetic reflex pathway.

Material and Methods

1. Preparation of animals

The experimental protocols were reviewed by the Committee on the Ethics of Animal Experiments in Tohoku University School of Medicine, and they were carried out in accordance with both the Guideline for Animal Experiments issued by the Tohoku University of Medicine and The law (No. 105) and Notification (No. 6) issued by the Japanese Government.

Twenty–four adult cats, unselected as to sex and of 2.0 to 3.4 kg body weight, were initially sedated with ketamine hydrochloride (30 mg/kg, i.m.) and then anaesthetized with a mixture of α–chloralose (50 mg/kg, i.v.) and urethane (100 mg/kg, i.v.). These anaesthetics were supplemented if and when necessary throughout the experiment. Local anaesthetic (2% Lidocaine, 1–2 ml) was always applied to the areas of the skin that were cut. One cephalic vein was cannulated to allow drug injection. The anaesthetized animals were intubated, paralyzed by intravenous injection of pancuronium bromide (Mioblock, Organon; 0.4 mg/kg initially, supplemented with 0.2 mg/kg per hour after testing the level of anaesthesia; see below) and artificially ventilated via the tracheal cannula with a mixture of 50 % air–50 % O_2. The ventilator (Shinano, Model SN–480–6, Tokyo, Japan) was sent to deliver at tidal volume of 10–12 cm^3/kg at a rate of 20 breaths/min. Continuous ventilation in this manner has been shown to maintain arterial blood pH at 7.4 ± 0.1, $PaCO_2$ at 31.3 ± 1.0 mmHg and PaO_2 at 240.0 ± 16.8 mmHg. Arterial blood was collected from the femoral artery every 1.5 h or so for the measurement of pH, $PaCO_2$ and PaO_2. Ringers solution was continuously infused at a rate of approximately 12 ml/h and 8.4% $NaHCO_3$ solution was added, if necessary, to maintain the arterial blood pH at the value given above. The criteria for maintenance of an adequate depth of anaesthesia were the persistence of miotic pupils and the absence of reflex elevation of heart rate and arterial blood pressure during stimulation of the lingual nerve (LN). If the depth of anaesthesia was considered inadequate, additional α–choloralose and urethane (i.e. intermittent doses of 5 mg/kg and 10 mg/kg i.v., respectively) was administered. Rectal temperature was maintained at 37–38℃ using a heating pad. In all experiments, the sympathetic trunks in

the neck were cut bilaterally prior to any stimulation to avoid the involvement of the cervical sympathetic nerves in any hypothalamic or reflex effects and to ensure that only parasympathetic effects were involved in the present study. At the end of the experiment, the cat was killed by an overdose (about 150 mg) of Nembutal.

2. Hypothalamic stimulation

After the head of the animal had been fixed in a stereotactic frame (Narishige, Tokyo, Japan), an electrode was placed in the anterior hypothalamus, the intended location being at stereotactic coordinates 13 mm rostral to the interaural line, 1 mm lateral to the midline and 2.5 mm below stereotactic zero (atlas of Snider & Niemer, 1961), unless otherwise stated. For this purpose, we used concentric bipolar electrodes, obtained from Inter Medical CO. Lt Tokyo, Japan, insulated with enamel except at the tip. The bare tip was 0.5 mm long and their impedance was 2 kQ. To produce moderate inhibitory responses with minimal brain damage, we usually used a 30 s train of rectangular square-wave pulses, generated by a Nihon Koden Model SEN-7103 stimulator through an isolationunit (Nihon Koden Model SS-202J, Tokyo, Japan), usually with an amplitude of 100-300 μA and a duration of 2 ms, at a frequency of 50 Hz, unless otherwise stated. The stimulating current was always set at an intensity less than that required to evoke an increase in arterial blood pressure by hypothalamic stimulation at the same site.

Reference sites were marked by passing 500 μA current for 30s. At the end of the experiment, the brain was perfused with saline followed by 10% formalin. The brain was then removed and fixed in 10% formalin and 5% potassium ferrocyanide (which produced a prussian blue spot at the reference site). The hypothalamic region was sectioned at 100 μm thickness on a freezing microtome and stained using the Nissl staining method. Histological study permitted identification of the site of stimulation with respect to the anatomical structures of the anterior hypothalamic area. The location of stimulated sites was recorded on representative coronal sections from the atlas of Snider & Niemer (1961).

3. Electrical stimulation of the Iingual nerve (LN)

To elicit a parasympathetic reflex vasodilatation in the lower lip and palate, the central cut end of the LN was electrically stimulated with a 20 Hz of 2 ms rectangular pulses at a frequency of 20 Hz and at supramaximal intensity (usually 30 V) as described before (Izumi & Karita, 1994, 1995; Karita & Izumi, 1993).

4. Chemical stimulation

Chemical stimulation within the hypothalamus was achieved using a solution of D,L-homocysteic acid (DLH, 1.0 M) in physiological saline. The pH of the DLH solution was adjusted to 7.4 by adding 5 N NaOH. A 120 μm needle (attached to a 1.0 μl syringe) was placed through the fixed guide cannula under stereotactic control at anterior hypothalamus area as described above (13 mm rostral to the interauralline, 1 mm lateral to the midline and 2.5 mm below stereotactic zero) and aliquots (0.2 μl of DLH solution were injected over periods of 10 s in order to keep the rate of injection approximately constant. All injection sites were marked by the injection of Methylene Blue in the same volume as that used for the injection of DLH and the marked sites were identified histologically as described above.

5. Measurement of the lip and palate blood flows, systemic arterial blood pressure and heart rate

Changes in blood flow at sites in the palate and in the mandibular lip adjacent to the canine tooth on either side were monitored using a laser Doppler flowmeter (LDF; Canon LC-1, Tokyo, Japan, or Advance ALF21R, Tokyo, Japan) as described before (Izumi & Karita, 1992, 1993; Karita & Izumi, 1995). The probe was placed against the lip or palate without exerting any pressure on the tissues. The present LDF values represent the blood flow in the superficial vessels in each tissue. Electrical calibration for zero blood flow was performed for all recordings. Several gains were selectable and the maximum output of a given gain level (defined electrically) was taken as 100%. The analog output of the equipment does not give absolute values, but shows relative changes in blood flow [for technical details and evaluation of the LDF method, see Stern et al. (1977)]. Output from the device was continuously displayed on an eight-channel chart recorder (Graphtec, Model W5000, Tokyo, Japan) at a speed of 10 mm/min. The blood flow changes were assessed by measuring the height of the response. Systemic arterial blood pressure was recorded from the femoralcatheter via a Statham pressure transducer. A tachograph (Nihon Koden Model AT-610G, Tokyo, Japan) triggered by the arterial pulse was used to monitor heart

rate.

6. Drugs

Picrotoxin was obtained from Wako Pure Chemical Ind. (Osaka, Japan). DLH was purchased from Tokyo Kasei Comp. (Tokyo, Japan) and lidocaine (2%) from Fujisawa Pharmaceutical Comp. (Osaka, Japan). All other chemicals were of reagent grade and were obtained from commercial sources.

7. Statistical analysis

All numerical data are given as the mean ± S.E. The significance of changes in responses was assessed using an analysis of variance (ANOVA) and a Contrast−tests. Differences were considered significant at the level $P < 0.05$. Data were analysed using a Macintosh Computer with StatView 4.5 and Super ANOVA.

Results

Mean values (± S.E.) for resting mean arterial blood pressure and heart rate were 107.3 ± 9.1 mmHg and 162.6 ± 6.5 beats/min in α−chloralose−urethane anaesthetized, paralyzed, artificially ventilated (50% air−50% O_2), vago−sympathectomized cats.

1. Modulation of parasympathetic reflex vasodilatation by electrical stimulation within the hypothalamus

Figure 1 includes a schematic representation of the experimental design showing the sites of electrical stimulation and of blood flow measurement (Ieft panel). The right−hand panel shows the effects of electrical stimulation within the anterior hypothalamus at 5 or 50 Hz on the reflex vasodilatation which was consistently evoked in the ipsilateral lower lip and palate by stimulation of the central cut end of the LN. The mechanism underlying such vasodilatation appears to involve an activation of a somato−parasympathetic reflex, as reported previously (Izumi & Karila, 1992, 1994, 1995; Karita & Izumi, 1993). Higher frequency (50 Hz) stimulation of the anterior hypothalamus markedly inhibited the reflex vasodilatation in the both lower lip and palate, but lower frequency (5 Hz) stimulation had no such effect. In these and the following experiments, hypothalamic stimulation was always begun 10 s before LN stimulation since this was found to give an inhibitory effect that was much greater than that induced by simultaneous stimulation of the hypothalamus and LN. No substantial difference was observed between the lower lip and palate in terms of the inhibitory effect on the parasympathetic reflex vasodilatation elicited by electrical stimulation of the hypothalamus and in subsequent experiments we concentrated on lip blood flow.

Step−by−step increases in the intensity of anterior hypothalamic stimulation progressively increased the inhibitory effect on the lip blood flow increase elicited by LN stimulation. Typical examples are shown in Fig. 2 and averaged

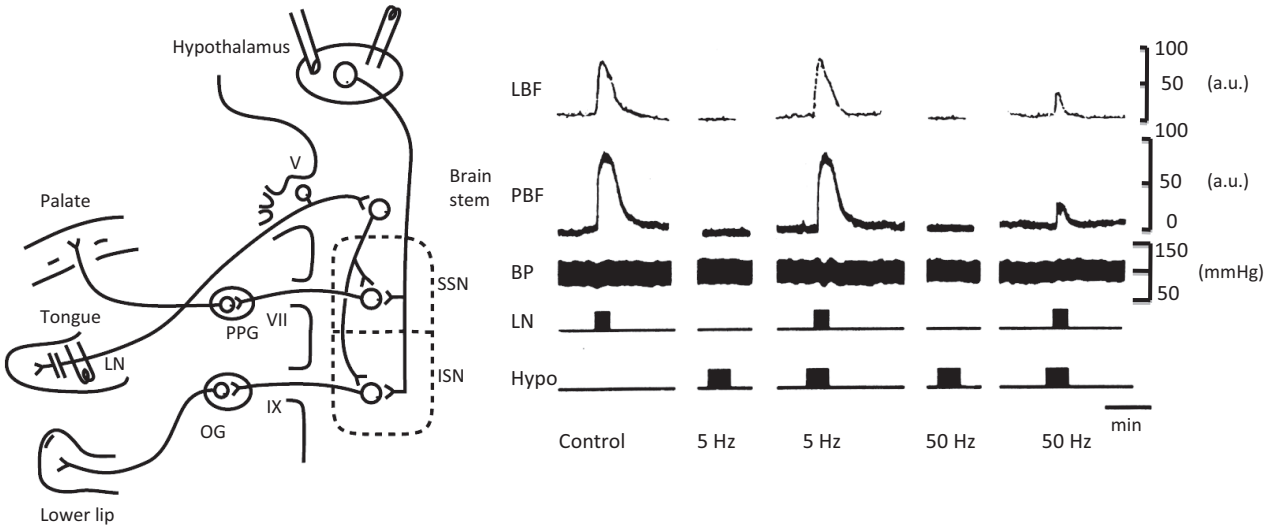

Fig. 1. Schematic representation of the sites of electrical and chemical stimulation and of blood flow measurement (Ieft panel), and the effects of electrical stimulation within anterior hypothalamus at 5 or 50 Hz on reflex vasodilatation in the ipsilateral lower lip and palate (right panel). Parameters for anterior hypothalamic (Hypo) and lingual nerve (LN) stimulation (indicated by bars) were, respectively : 2 ms pulses, 5 or 50 Hz, 200 μA for 30 sec and 30 V, 2 ms, 20 Hz for 20 sec. Ordinate, lip (LBF) and palate blood flow (PBF in arbitrary units (a.u.) and systemic arterial blood pressure (BP, mmHg). Abbreviations : SSN, superior salivatory nucleus ; ISN, inferior salivatory nucleus ; LN, lingual nerve ; PPG, pterygopalatine ganglion ; OG, otic ganglion ; V, trigeminal nerve root ; VII , facial nerve root ; IX, glossopharyngeal nerve root.

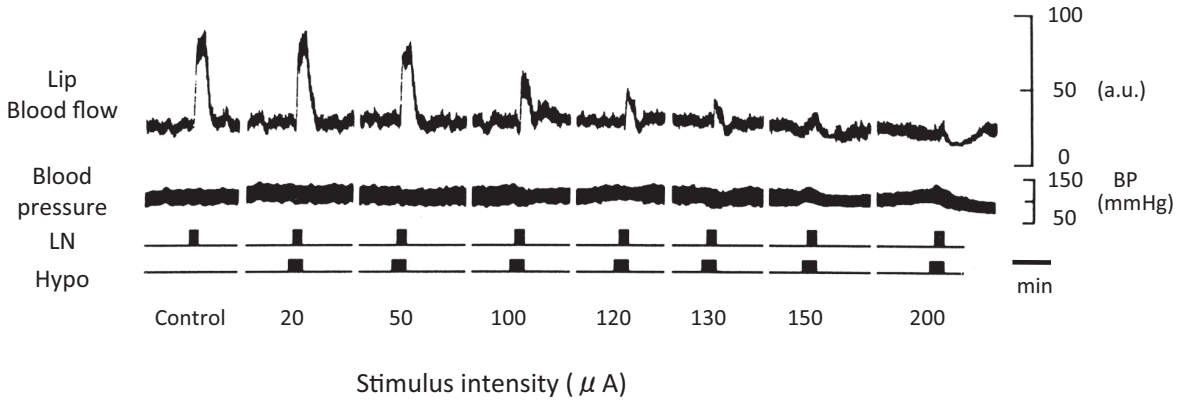

Fig. 2. Intensity−response relationship for inhibitory effect of anterior hypothalamic stimulation on the lip blood flow increase reflexly elicited by electrical stimulation of the lingual nerve (LN) in vago−sympathectomized anaesthetized cats. Parameters for anterior hypothalamic (Hypo) and LN stimulation (indicated by bars) were, respectively: 2 ms pulses, 50 Hz, 20 − 200 μA for 30 s and 30 V, 2 ms, 20 Hz for 20 s. Ordinate, lip blood flow in arbitrary units (a.u.) and systemic arterial blood pressure (mmHg).

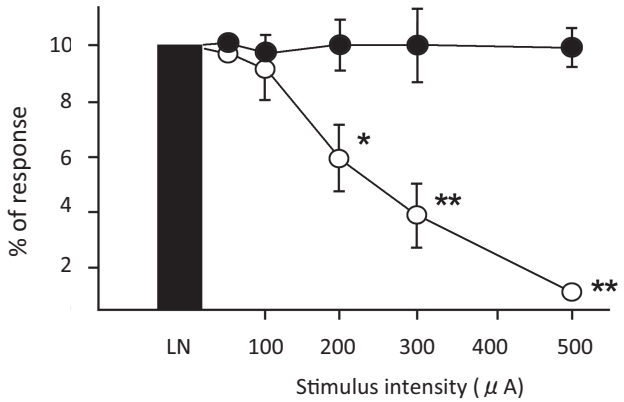

Fig. 3. Averaged data for effect of anterior hypothalamic stimulation at various stimulus intensities (50−500 μA) in the presence (open circles) and absence (closed circles) of picrotoxin on the blood flow increase in lower lip (LBF reflexly elicited by electrical stimulation of the lingual nerve (LN) in vago−sympathectomized anaesthetized cats. Picrotoxin (1 mg/kg, i.v.) was administered 10 − 20 min before electrical stimulation was repeated. The anterior hypothalamus was electrically stimulated (2 ms pulses at 50 Hz for 30 s with stimulus intensity (50 − 500 μA) beginning 10 sec before LN stimulation (2 ms pulses at 20 Hz for 20 s with supramaximal intensity). Ordinate, value of LBF increase expressed as a percentage of the increase elicited by electrical stimulation of the LN alone. Values shown are means ± S.E. from 6 animals. Statistical significance was assessed using analysis of variance (ANOVA) for repeated measurement folowed by a contrast test for significance of difference. *P < 0.01 ; **,P < 0.001 vs response elicited by LN stimulation alone.

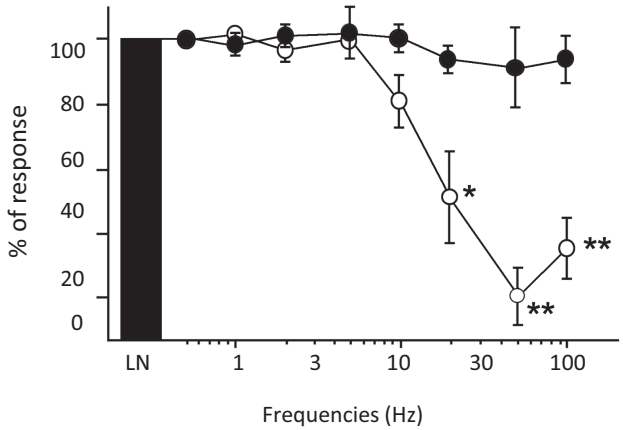

Fig. 4. Averaged data for effect of anterior hypothalamic stimulation at various stimulus frequencies (0.5 − 100 Hz)in the presence (open circles) and absence (c1osed circles) of picrotoxin on the reflex blood flow increase in lower lip (LBF) elicited by electrical stimulation of the Iingual nerve (LN) in vago−sympathectomized anaesthetized cats. Picrotoxin (1 mg/kg, i.v.) was administered 10−20 min prior to repeated electrical stimulation of the LN. The anterior hypothalamus was electrically stimulated (2 ms pulses at 50 Hz for 30 s with stimulus intensity 0.1−0.2 mA) beginning 10 sec before LN stimulation (2 ms pulses at 20 Hz, for 20 s with supramaximal intensity). Ordinate, value of LBF increase expressed as a percentage of the increase elicited by electrical stimulation of the LN alone. Values shown are means ± S.E. from 6 animals. Statistical significance was assessed using analysis of variance (ANOVA) for repeated measurement followed by a contrast test for significance of difference. *, P < 0.05; **, P < 0.01 vs response elicited by LN stimulation alone.

data in the presence and absence of picrotoxin in Fig. 3. Hypothalamic stimulation with currents of less than 130 μA did not cause any increase in arterial blood pressure, but intensities of more than 150 μA did elicit such an increase. Although the threshold intensity needed to elicit a rise in arterial blood pressure varied from animal to animal, the current intensity needed for the present inhibitory effect was always below that threshold intensity. This suggests that hypothalamic stimulation at the level that induced the inhibitory effect under the present stimulus conditions did not itself cause any significant cardiovascular effects and we observed no other autonomic responses, such as pupillary dilatation and piloerection.

As shown in Fig. 4, the inhibitory effect of hypothalamic stimulation was frequency−dependent at frequencies of more than about 10 Hz, while there was no inhibitory effect at frequencies of 0.5−5 Hz. The optimum stimulus frequency for eliciting the inhibitory effect was 50 Hz (Fig. 4).

Prior administration of picrotoxin, a relatively specific antagonist of $GABA_A$ receptors, (Bloom, 1990) at dose of 1mg /kg, i.v. completely abolished the inhibitory effect induced by electrical stimulation within the anterior hypothalamus at any of the stimulus intensities (Fig. 3) or frequencies used (Fig. 4). This suggests that GABAergic synapses might be

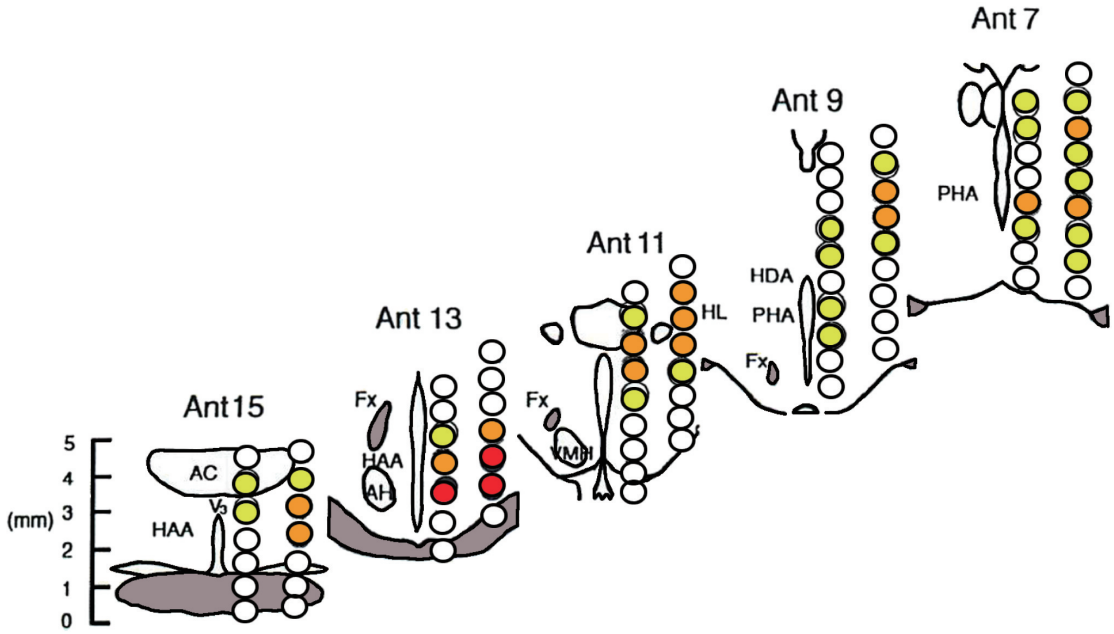

Fig. 5. Representative transverse sections of hypothalamus (from 7 to 15 mm rostral to interaural line) showing location of sites at which an inhibitory response was evoked by electrical stimulation (see Figs. 1–4). ●, sites at which stimulation reduced the blood flow increase by more than 75 % ; ● , more than 50 % ; ●, more than 25 % and ○, sites from which no such inhibitory response was elicited. Calibration scale in mm. AC, anterior commissure ; AH, anterior hypothalamic nucleus ; FX, fornix ; HAA, anterior hypothalamic area ; HDA, dorsal hypothalamic area ; H L, lateral hypothalamus ; OC, optic chiasma ; PHA, posterior hypothalamic area ; VMH, ventromedial hypothalamic nucleus ; V_3 third ventricle.

involved in relaying the inhibitory effect from the hypothalamus to the parasympathetic reflex vasodilator mechanism. However, this reagent must be used with caution, since the disinhibitory effect of picrotoxin had all but disappeared within 90 min of its administration and since administration of picrotoxin itself sometimes (5 out of 12 tests) caused a vasodilator effect in the lower lip. A similar effect is observed with pentylenetetrazole, another GABA receptor antagonist (Izumi et al., 1995).

2. Sites from which hypothalamic inhibition was elicited

Figure 5 shows the location of histologically verified sites in the periventricular and medial zones of the hypothalamus at which electrical stimulation elicited a inhibitory effect on the reflex increase in lip blood flow. As electrical stimulation within the lateral zone of the hypothalamus, using a similar stimulus current, evoked an increase in arterial blood pressure, we did not try to examine the effect of stimulation in this region on the parasympathetic reflex mechanism. The effective stimulation sites lay in a region extending from anterior 15 mm to anterior 7 mm; the most effective sites all being at anterior 13 mm.

3. Modulation of parasympathetic reflex vasodilatation by microinjection of D, L–homocysteic acid (DLH)

Figure 6 shows the effect of microinjection into the anterior hypothalamus of D, L–homocysteic acid (DLH, 1 M, 0.2 µl), an excitatory amino acid , on the lip blood flow Increase reflexly elicited by LN stimulation. A statisticallY significant attenuation of the control response was observed on microinjection of DLH (reduced by 27.4 ± 10.9%, n = 6; P< 0.05 using an ANOVA test), but not on microinjection of saline alone.

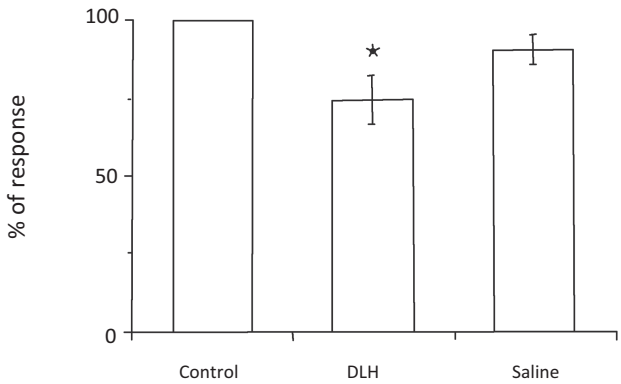

Fig. 6. Effect of microinjection of 0.2 µl of 1 M D,L–homocysteic acid (DLH) and 0.2 µl physiological saline (saline) into the anterior hypothalamus [anterior, 13 mm ; lateral , 1 mm ; 2.5 m below stereotactic zero (Snider and Niemer, 1961)] on the blood flow increase in the lower lip reftexly elicited by electrical stimulation of the lingual nerve alone (control). Values shown are means ± S.E. from 6 animals. Statistical significance was assessed using analysis of variance (ANOVA). *, P < 0.05 vs control.

Discussion

The initial aim of the present study was to determine if any modulation could be evoked from the hypothalamus of

the parasympathetically mediated reflex vasodilatation in lower lip and palate evoked by electrical stimulation of the LN in vago−sympathectomized cats. For this study, α−chloralose and urethane were chosen as the anaesthetic agents because they have considerably fewer depressant effect on central nervous structures than barbiturates or other commonly used general anaesthetics (Brown & Hilton , 1956; Price, 1960; Izumi et al., 1997, 1999; Ito et al., 1998; Mizuta et al., 2006). Our considerable experience with this anaesthetic regime has shown that, with careful attention to dose and method of administration, it produces a stable and consistent level of anaesthesia for an extended period of time. Pancuroniium was used to allow control ventilation and to prevent any muscle movement during periods of brain stimulation, We have found that, when used in the doses employed in our study, pancuronium produces immobilization without any persistent effects on the cardiovascular system.

Electrical stimulation of the anterior hypothalamus with an intensity in the range 50−500 μA elicited an attenuation of the parasympathetically mediated reflex vasodilatation in the lower lip and palate (Fig. 1). The effect was the intensity −dependent (Fig. 3) and the optimal stimulus frequency was 50 Hz (Fig. 4). This optimum frequency was quite consistent with those reported to induce (i) hypothalamic inhibition of the baroreceptor reflex cardiovascular response (Coote et al., 1979; Jordan et al., 1988; Mifflin et al., 1988), (ii) the hypothalamic depressor response (Hilton & Spyer, 1971) and (iii) the defence−alerting response, including muscle vasodilatation, in response to hypothalamic stimulation (Abrahams et al., 1960).

The question of stimulus spread is quite important. It might be thought that spread to sensory structures (thalamus) mediating pain sensation might account for the results. However, the highly localized distribution of the most effective electrode positions within the anterior hypothalamus argues against this conclusion. It was necessary to increase the stimulus intensity three to four times to elicit similar inhibitory effects at points only 2 mm removed from the optimal area. Also, Figure 5 shows that sites in the optic chiasma and optic tract were consistently ineffective, as were those dorsal to (i.e. on the thalamic side) of the effective sites. It therefore seems unlikely that stimulus spread and pain sensation contributed significantly to the results.

A number of factors had to be considered as possible contributors to the inhibitory effect. Hypothalamic stimulation is known to release adrenaline from the adrenal medulla, as well as ADH from the pituitary gland, and to influence preganglionic sympathetic neurons (Harris & Loewy, 1990). However, it is unlikely that the present inhibitory effect is due to a release of these humoral substances or to any sympathetically mediated action, since (i) the inhibitory effect occurred in animals sympathectomized in the neck and was unaffected by spinal section at the level of C_2 (unpublished observation), (ii) electrical stimulation of the anterior hypothalamus with the stimulus characteristics used in the present study elicited an inhibitory effect without any increase in arterial blood pressure, (iii) the latency is too short for a humoral effect of that type. Thus, these results suggest that the inhibitory effect is due to a direct neural effect exerted at some sites in the reflex arc for the parasympathetic vasodilatation. From the present results, a likely inference is that the anterior hypothalamus sends inhibitory fibers to the preganglionic parasympathetic neurons situated within the inferior and superior salivatory nuclei. However, as discussed more fully below, it remains obscure whether or not the inhibitory responses derive from specific anterior hypothalamic neurons alone or from some pathways traversing this area, since the degree of inhibition induced by the microinjection of DLH was much smaller (20−30%)(Fig. 6) than that elicited by electrical stimulation of the anterior hypothalamus (Figs. 3 & 4).

Picrotoxin blocked the hypothalamic inhibition of the reflex vasodilator response in the lower lip (Figs. 3 & 4), suggesting that the hypothalamic inhibitory effect might be mediated by GABA−like effects, possibly within the superior and inferior salivatory nuclei, which presumably correspond to the reticular area dorsal to the facial nucleus termed DFA (dorsal to the facial nucleus) by Kuo et al. (1987). Electrical and chemical (glutamate) stimulation of DFA has been reported to increase regional blood flow in extracranial tissues by activating parasympathetic preganglionic neurons, without induced changes in systemic arterial blood pressure (Chyi et al., 1995; Kuo et al., 1995). This disinhibitory effect of picrotoxin may be similar to those observed at other sites which suggest that GABAergic synapses may be involved in the modulation of cardiorespiratory control processes. For example, general anaesthetics depress evoked potentials in the thalamus and hypothalamus and may convert responses to somatic afferent fiber stimulation from depres-

sor to pressor ones. The underlying mechanism has been said to be due to an enhancement or imitation of the action of GABA at central vasomotor synapses (Lalley, 1980; Price, 1960). Interestingly, Jordan et al. (1988), who made intracellular recordings from neurons receiving baroreceptor inputs, have reported the possibility that GABA may act as an inhibitory transmitter mediating the inhibitory actions of hypothalamic defence area stimulation on neurons of the nucleus tractus solitarius.

In our mapping study, the anterior hypothalamus was found to be the most effective area, among the periventricular hypothalamic regions examined, in eliciting the inhibitory effect, although other hypothalamic areas had a moderate inhibitory effect (Fig. 5). The finding that stimulation within a relativeIy wide area of the hypothalamus evoked the inhibitory effect might reflect either the existence of reciprocal neural links between the various hypothalamic nuclei (see review by Luiten et al., 1987) or to activation of axons travelling to or from a specific inhibitory locus or, indeed, through it.

A number of cardiovascular responses (pressor, depressor, defence responses etc.) can be evoked from the hypothalamus (see review by Jordan, 1990). Hypothalamic inhibition of the baroreceptor reflex cardiovascular response is well-attested (see review by Spyer, 1990) and can be evoked from an area that might include our inhibitory area. This may indicate that more than one type of response can be evoked from a single area. This could occur if a particular area has, say, a rather general inhibitory role, or if axons belonging to different neural systems all pass through one and the same area.

Only comparatively few neural pathways have been positively shown to run between the hypothalamus and preganglionic parasympathetic neurons. These are (i) the pathway from the paraventricular nucleus to the Edinger-Westphal component of the oculomotor complex and to the dorsal vagal complex, which consists of preganglionic neurons of the dorsal motor nucleus of the vagus (see reviews by Luiten et al., 1987; Swanson, 1987), and (ii) the lateral hypothalamic input to the superior salivary nucleus (Hosoya et al., 1983).

Although electrical stimulation has played an important role in the identification of the specific brain regions which subserve emotional behavioural and other responses, a major disadvantage is that both cell bodies and axons of passage are excited by the stimulating current (Rank, 1975). Therefore, it is not clear whether the inhibitory effect elicited by electrical stimulation is due to excitation of cell bodies or of fibers of passage.

For this reason, it is useful to employ another method of stimulating central neurons, by introducing an excitatory amino acid (e.g. L-glutamic acid or DLH) into their immediate environment. As shown in Fig. 6, a statistically significant inhibitory effect was observed after microinjection of DLH, suggesting that cell bodies in the anterior hypothalamus are responsible, at least in part, for the inhibitory action. However, the inhibitory effect of DLH was much smaller than that caused by electrical stimulation of the anterior hypothalamus. There are a number of factors that may underlie this discrepancy. One is the responsiveness of the cell bodies to DLH; others are the density of the relevant cell bodies within the anterior hypothalamus, electrical (but not chemical) excitation of axons to or from the anterior hypothalamus, different degrees of spread of the two types of stimulus, and the possible involvement of fibers of passage. At this stage, it is not possible to locate with any precision a "DLH-effective zone" (in other words, to be sure of the location of the cell-bodies. responsible for the inhibitory influence over the reflex response). This is because (i) fewer sites were stimulated with DLH than by electrical stimulation (moving the guide cannula to several sites would cause too much damage to the brain), and the DLH-effective sites were scattered around the anterior hypothalamus (but not outside it), and (ii) the inhibitory effect of DLH was of about the same magnitude (20-30%, see Fig. 6) at each of the effective sites stimulated. For that reason, in contrast to the situation with electrical stimulation, there was no possibility of identifying an area in which stimulation was particularly effective within a less-effective zone. At this stage, we can only say that, as injection of DLH into the anterior hypothalamus was effective in evoking the inhibition, the anterior hypothalamus presumably contain the cell-bodies of neurons that mediate this effect is not evoked merely by stimulation of fibers of passage.

In the present experiments, electrical stimulation of the anterior hypothalamus elicited the inhibitory effect described here without raising arterial blood pressure. Thus, the inhibitory effect we evoked from the anterior hypothalamic area probably has no physiological association with the defence reaction, which involves a pronounced rise in blood pressure, even though the defence area appears to be close to

our inhibitory area at the anterior hypothalamic level. Further support for this assumption is that neither electrical stimulation of the present type nor chemical stimulation (DLH) of anterior hypothalamus induced any other autonomic effects, such as pupilary dilatation and piloerection (characteristic components of the defence reaction). However, it must be admitted that the sympathetic innervation of the iris would have been severed by our cervical sympathectomy. This is the first detailed study showing an influence of the anterior hypothalamus over non−vagal parasympathetic reflex mechanisms involving the oro−facial areas of the cat. Further study will be needed to determine if the effect can be ascribed to a particular anatomical entity and to examine its physiological significance.

References

Abrahams VC, Hilton SM & Zbrozyna A. Active muscle vasodilatation produced by stimulation of the brain stem : its significance in the defence reaction. J Physiol 154 : 491–513, 1960.

Abrahams VC & Hilton SM. The Role of Active Muscle Vasodilatation in the Alerting Stage of the Defence Reaction. J Physiol 171 : 189–202, 1964.

Bandler R. Induction of 'rage' following microinjections of glutamate into midbrain but not hypothalamus of cats. Neurosci Lett 30 : 183–188, 1982.

Bloom FE. Neurohumoral transmission and the central nervous system. In : Gilman AG, Rall TW, Nies AS & Taylor, editors. P. Goodman and Gilman's The Pharmacological Basis of Therapeutics, Eighth edition, New York : Pergamon Press, 1990, pp244–268.

Brown RV & Hilton JG. The effectiveness of the baroreceptor reflexes under different anesthetics. J Pharmacol Exp Ther 118 : 198–203, 1956.

Chyi T, Cheng V, Chai CY & Kuo JS. Vasodilatation produced by stimulation of parvocellular reticular formation in the medulla of anesthetized−decerebrate cats. J Auton Nerv Syst 56 : 69–74, 1995.

Coote JH, Hilton SM & Perez−Gonzalez JF. Inhibition of the baroreceptor reflex on stimulation in the brain stem defence centre. J Physiol 288 : 549–560, 1979.

Gibbins IL, Brayden JE & Bevan JA. Perivascular nerves with immunoreactivity to vasoactive intestinal polypeptide in cephalic arteries of the cat : distribution, possible origins and functional implications. Neuroscience 13 : 1327–1346, 1984.

Harris MC & Loewy AD. Neural regulation of vasopressin containing hypothalamic neurons and the role of vasopressin in cardiovascular function. In : Loewy AD & Spyer KM, editors. Central Regulation of Autonomic Functions, New York, Oxford : Oxford University Press, 1990, pp224–246.

Hilton SM & Redfern WS. A search for brain stem cell groups integrating the defence reaction in the rat. J Physiol 378 : 213−228, 1986.

Hilton SM & Spyer KM. Participation of the anterior hypothalamus in the baroreceptor reflex. J Physiol 218 : 271–293, 1971.

Hosoya Y, Matsushita M & Sugiura Y. A direct hypothalamic projection to the superior salivatory nucleus neurons in the rat. A study using anterograde autoradiographic and retrograde HRP methods. Brain Res 266 : 329–333, 1983.

Ishii H, Niioka T & Izumi H. Vagal visceral inputs to the nucleus of the solitary tract : involvement in a parasympathetic reflex vasodilator pathway in the rat masseter muscle. Brain Res 1312 : 41−53, 2009.

Ishii H, Niioka T & Izumi H. Parasympathetic reflex vasodilatation in the masseter muscle compensates for carotid hypoperfusion during the vagus−mediated depressor response. Brain Res 1370 : 145−153, 2011.

Ishii H, Niioka T, Sudo E & Izumi H. Evidence for parasympathetic vasodilator fibres in the rat masseter muscle. J Physiol 569 : 617−629, 2005.

Ishii H, Niioka T, Watanabe H & Izumi H. Inhibitory effects of excess sympathetic activity on parasympathetic vasodilation in the rat masseter muscle. Am J Physiol 293 : R729−736, 2007.

Ito Y, Izumi H, Sato M, Karita K & Iwatsuki N. Suppression of parasympathetic reflex vasodilatation in the lower lip of the cat by isoflurane, propofol, ketamine and pentobarbital : implications for mechanisms underlying the production of anaesthesia. Br J Anaesth 81 : 563−568, 1998.

Izumi H & Karita K. Vasodilator responses following intracranial stimulation of the trigeminal, facial and glossopharyngeal nerves in the cat gingiva. Brain Res 560 : 71−75, 1991.

Izumi H & Karita K. Somatosensory stimulation causes autonomic vasodilatation in cat lip. J Physiol 450 : 191−202, 1992.

Izumi H & Karita K. Innervation of the cat lip by two groups of parasympathetic vasodilator fibres. J Physiol 465 : 501−512, 1993.

Izumi H & Karita K. Parasympathetic−mediated reflex salivation and vasodilatation in the cat submandibular gland. Am J Physiol 267 : R747−753, 1994.

Izumi H & Karita K. Salivary secretion in cat submandibular gland mediated by chorda tympani afferents. Am J Physiol 268 : R438−444, 1995.

Izumi H, Takahashi H & Karita K. Pentylenetetrazole−induced parasympathetic blood flow increase in the lower lip of the cat. Eur J Pharmacol 273 : 299−302, 1995.

Izumi H, Ito Y, Sato M, Karita K & Iwatsuki N. Effects of inhalation anesthetics on parasympathetic reflex vasodilation in the lower lip and palate of the cat. Am J Physiol 273 : R168−174, 1997.

Izumi H & Ito Y. Correlation between degree of inhibition of parasympathetic reflex vasodilation and MAC value for various inhalation anesthetics. Gen Pharmacol 32 : 689−693, 1999.

Jordan D, Mifflin SW & Spyer KM. Hypothalamic inhibition of neurones in the nucleus tractus solitarius of the cat is GABA mediated. J Physiol 399 : 389−404, 1988.

Jordan D. Autonomic changes in affective behavior. In : Loewy AD & Spyer KM. Central, editors. Regulation of Autonomic Functions, New York, Oxford : Oxford : University Press 1990, pp349−366.

Karita K & Izumi H. Dual afferent pathways of vasodilator reflex induced by lingual stimulation in the cat. J Auton Nerv Syst 45 : 235−240, 1993.

Karita K & Izumi H. Effect of baseline vascular tone on vasomotor responses in cat lip. J Physiol 482 : 679−685, 1995.

Koeda S, Ishii H, Kuchiiwa S & Izumi H. Role of the spinal trigeminal nucleus in the rat autonomic reflex. Arch Oral Biol 54 : 1136−1142, 2009.

Kojima K, Hirata K, Higuchi S, Akazawa K & Tashiro N. The relation of emotional behavior to plasma catecholamines, cortisol and ventricular arrhythmia. J Auton Nerv Syst 55 : 57–64, 1995.

Kuo JS, Chyi T, Yang MC & Chai CY. Changes in intra– and extracranial tissue blood flow upon stimulation of a reticular area dorsal to the facial nucleus in cats. Clin Exp Pharmacol Physiol 22 : 87–93, 1995.

Kuo JS, Wang MR, Liu RH, Yu CY, Chiang BN & Chai CY. Reduction of common carotid resistance upon stimulation of an area dorsal to the facial nucleus of cats. Brain Res 417 : 181–184, 1987.

Lalley PM. Inhibition of depressor cardiovascular reflexes by a derivative of gamma–aminobutyric acid (GABA) and by general anesthetics with suspected GABA–mimetic effects. J Pharmacol Exp Ther 215 : 418–425, 1980.

Luiten PG, ter Horst GJ & Steffens AB. The hypothalamus, intrinsic connections and outflow pathways to the endocrine system in relation to the control of feeding and metabolism. Prog Neurobiol 28 : 1–54, 1987.

Mifflin SW, Spyer KM & Withington–Wray DJ. Baroreceptor inputs to the nucleus tractus solitarius in the cat : modulation by the hypothalamus. J Physiol 399 : 369–387, 1988.

Mizuta K & Izumi H. Bulbar pathway for contralateral lingual nerve–evoked reflex vasodilatation in cat palate. Brain Res 1020 : 86–94, 2004.

Mizuta K, Kuchiiwa S, Saito T, Mayanagi H, Karita K & Izumi H. Involvement of trigeminal spinal nucleus in parasympathetic reflex vasodilatation in cat lower lip. Am J Physiol Regul Integr Comp Physiol 282 : R492–500, 2002.

Mizuta K, Mizuta F, Takahashi M, Ishii H, Niioka T & Izumi H. Effects of isoflurane on parasympathetic vasodilatation in the rat submandibular gland. J Dent Res 85 : 379–383, 2006.

Niioka T, Ishii H & Izumi H. Involvement of vasoactive intestinal polypeptide in the parasympathetic vasodilatation of the rat masseter muscle. Arch Oral Biol 54 : 909–916, 2009.

Price HL. General anesthesia and circulatory homeostasis. Physiol Rev 40 : 187–218, 1960.

Rank JB. Which elements are excited in electrical stimulation of mammalian central nervous system : a review. Brain Res 98, 1975, pp417–440.

Snider RS & Nimer WT. A stereotaxic Atlas of the Cat Brain. Chicago and London : The University of Chicago Press, 1961.

Sakurai Y, Echigo S, Kuchiiwa S & Izumi H. Lingual nerve stimulation–induced c–Fos expression in the trigeminal spinal nucleus. Dent. J. Health Sci. Univ. Hokkaido 25 : 97–107, 2007.

Spyer KM. The central nervous organization of reflex circulatory control. In : Loewy AD & Spyer KM, edioers. Central Regulation of Autonomic Functions. New York : Oxford : Oxford University Press, 1990, pp168–188.

Stern MD, Lappe DL, Bowen PD, Chimosky JE, Holloway GA Jr., Keiser HR & Bowman RL. Continuous measurement of tissue blood flow by laser–Doppler spectroscopy. Am J Physiol 232 : H 441–448, 1977.

Swanson LW. The hypothalamus. In : Bjorklund A, Hokfelt T, Swanson LW, editors. Handbook of Chemical Neuroanatomy, Vol. 5, Integrated Systems of the CNS, Part 1, Hypothalamus, Hippocampus, Amygdala, Retina. Amsterdam–New York–Oxford : Elsevier, 1987, pp1–124.

Yardley CP & Hilton SM. The hypothalamic and brainstem areas from which the cardiovascular and behavioural components of the defence reaction are elicited in the rat. J Auton Nerv Syst 15 : 227–244, 1986.

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