Localization of Sympathetic, Parasympathetic and Sensory Neurons Innervating the Heart of the Beijing Duck by Means of the Retrograde Transport of Horseradish Peroxidase

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ABSTRACT. Sympathetic, parasympathetic and sensory neurons were labeled by injections of horseradish peroxidase into various regions of the heart in 33 Beijing ducks. Sympathetic postganglionic neurons innervating the heart were located in the paravertebral ganglia C15 (C16 is the last cervical segment in the duck) to T3, especially in the ganglion T1. The coronary sulcus and ventricle were more abundantly innervated by sympathetic neurons than the atrium. The left side of the heart was preferentially innervated by sympathetic postganglionic neurons in the left side of paravertebral ganglia but the right side of the heart were equally supplied from the right and left ganglia. Within the medulla oblongata, the number of labeled vagal preganglionic neurons in the nucleus ambiguus was much greater than that in the dorsal motor nucleus of the vagus nerve. Labeled neurons of the nucleus ambiguus were found in many ducks injected into the coronary sulcus. Cardiac sensory neurons were observed in the dorsal root ganglia C15 to T2 (highest in the ganglion T1) and in the nodose and jugular ganglia of the vagus nerve. These labeled neurons probably form the afferent and efferent limbs of cardiac reflexes and control circulation in the Beijing duck.—**KEY WORDS:** autonomic nervous system, Beijing duck, heart, parasympathetic neuron, sympathetic neuron.

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In the heart of birds, like that of mammals, heart rate is set by the interplay of sympathetic and parasympathetic influences on the pacemaker. Stimulation of the sympathetic cardiac nerve typically increases heart rate, whereas stimulation of the parasympathetic vagus nerve reduces it [17]. There seems to be considerable species variation in the amount of vagal restraint of the heart. Species with a large heart in relation to body size like pigeons, most carnivorous birds, gulls, and ducks have a marked vagal tonus to the heart, while for species like chickens the tonic vagal activity is considerably smaller. This generalization appears to be true at least for the duck and chicken, since bilateral vagotomy of the duck increases heart rate by 65–200%, whereas the same procedure in the chicken increases heart rate by only 20–36% [17]. These species differences are related to the activity pattern of the birds in normal life.

The distribution of sympathetic, parasympathetic and sensory neurons innervating the avian heart was only studied in the pigeon using retrograde axonal transport of horseradish peroxidase (HRP) [3], electrical stimulation [7, 25] and retrograde degeneration [8, 20]. These studies demonstrated that sympathetic postganglionic neurons were located mainly in the paravertebral ganglion C14 (the last cervical segment in the pigeon), parasympathetic preganglionic neurons were observed in the dorsal motor nucleus of the vagus nerve and sensory neurons were found in the dorsal root ganglia C13 and C14. However, using the fragment C of tetanus toxin as the transport marker molecule, it was demonstrated that the majority of

parasympathetic preganglionic neurons innervating the heart of the pigeon were located in the nucleus ambiguus [4].

Recently, distributions of the cardiac plexus and cardiac ganglia were gross-anatomically studied in the Beijing duck [5]. Six cardiac plexuses and cardiac ganglia arising from the sympathetic trunk and the vagus nerve made the particular pattern of the autonomic nerve networks on the heart of the duck. However, few transport studies have been carried out in the duck. The present study aims at elucidating the precise anatomical distribution of sympathetic postganglionic, parasympathetic preganglionic and sensory neurons innervating each cardiac region of the Beijing duck using retrograde axonal transport of HRP.

MATERIALS AND METHODS

Thirty-three Beijing ducks (*Anas platyrhynchos* var. *domesticus*) weighing 1–2 kg (1–2 months old) were used in this study. Under anesthesia with 30% solution of urethane (0.8 g/kg, i.v.), the heart was exposed and 20% HRP (Sigma, Type VI) was injected into the subepicardium in 1 or 3 regions of the right and left atria, right and left ventricles and right coronary sulcus (Table 1). Injections of HRP were performed at 5 points (5 μ l/point) in each cardiac region for 10–25 min. After a postinjection survival time of 30–36 hr, ducks were deeply anesthetized with 30% solution of urethane (1 g/kg, i.v.) and perfused with 0.75% saline (40°C) through the left ventricle, followed by the fixative consisting of 2% paraformaldehyde and 1.25%

Table 1. The number of labeled neurons in the paravertebral, dorsal root, nodose and jugular ganglia and in the medulla oblongata after injections of HRP into the heart of the Beijing duck

Duck No.	Injection site	Right paravertebral ganglia					Left paravertebral ganglia					Right	Left	Right	Left	Right dorsal root ganglia				Left dorsal root ganglia				Right	Left	Right	Left
		C15	C16	T1	T2	T3	C15	C16	T1	T2	T3	DMNV	DMNV	NA	NA	C15	C16	T1	T2	C15	C16	T1	T2	NG	NG	JG	JG
D13	RA	–	–	3	–	–	–	–	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
D50	RA	–	–	8	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
D51	RA	–	–	2	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
D61	RA	–	–	3	2	–	–	–	–	3	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	
D34	RCS	–	34	336	177	–	–	48	194	15	1	–	–	3	3	–	2	29	2	–	4	4	4	–	1	–	
D35	RCS	–	–	–	–	–	–	–	–	–	–	–	1	4	3	–	–	–	–	–	–	–	–	–	–	–	
D36	RCS	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	
D41	RCS	–	–	37	29	–	–	–	39	18	–	–	–	–	–	–	–	1	–	–	–	–	2	2	–	–	
D63	RCS	–	3	20	2	–	–	13	37	10	–	6	–	2	1	–	–	–	–	–	–	–	–	19	–	–	
D64	RCS	–	25	61	1	–	–	12	14	2	–	11	15	9	8	–	1	1	1	–	3	7	–	–	–	–	
D20	RA+RCS	–	–	4	1	–	–	8	34	6	–	1	2	6	6	–	–	–	–	–	–	–	–	6	–	11	
D22	RA+RCS	–	–	–	–	–	–	–	–	–	–	–	2	1	2	–	–	–	–	–	–	–	–	–	–	–	
D23	RA+RCS	–	–	–	–	–	–	–	–	–	–	–	1	–	1	–	–	–	–	4	20	–	–	–	–	–	
D25	RA+RCS	–	11	32	3	–	–	2	36	4	–	–	–	5	2	–	–	1	–	–	–	–	2	4	–	–	
D26	RA+RCS	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	2	1	–	–	
D27	RA+RCS	–	–	–	–	–	–	–	–	–	–	–	–	2	4	–	–	–	–	–	–	–	–	–	–	7	
D29	RA+RCS	–	–	–	–	–	–	–	–	–	–	–	1	3	4	–	–	–	–	–	–	–	–	1	–	–	
D39	RA+RCS	–	13	13	30	–	–	6	4	10	–	–	–	–	–	–	–	1	–	–	–	1	3	–	–	–	
D40	RA+RCS	–	6	28	1	–	–	1	175	–	–	–	–	–	–	–	–	3	–	–	–	1	–	2	–	–	
D4	RV	–	1	27	6	–	–	6	12	–	–	–	–	–	–	–	–	–	–	–	4	–	8	–	–	–	
D8	RV	–	10	18	4	–	–	4	12	3	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	
D9	RV	–	7	62	–	–	–	15	137	9	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	
D52	RV	–	33	118	23	–	–	22	123	59	–	–	–	–	–	–	16	9	3	–	6	23	7	–	3	–	
D42	All R	–	3	83	8	–	–	11	90	20	–	7	7	15	16	–	–	–	3	–	–	–	4	2	8	–	
D31	LA	–	–	–	–	–	–	–	13	5	–	–	–	–	–	–	–	–	–	–	–	–	4	–	–	–	
D46	LA	–	–	3	–	–	–	–	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
D55	LA	–	6	10	–	–	15	4	15	–	–	–	–	–	–	–	–	1	–	2	–	5	–	–	–	–	
D57	LA	–	–	–	–	–	–	1	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
D33	LV	–	1	13	3	–	–	4	24	171	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	
D44	LV	–	84	77	3	–	–	100	331	171	–	–	–	–	–	–	–	–	–	8	17	24	–	2	–	–	
D45	LV	–	–	8	–	–	–	1	13	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	
D47	LV	–	–	31	7	–	–	6	105	45	2	2	–	4	3	–	–	–	–	–	–	–	2	–	–	–	
D48	LV	–	–	–	–	–	–	–	–	–	–	–	–	10	5	–	–	–	–	–	–	2	–	–	–	–	

All R: RA+RCS+RV; DMNV: dorsal motor nucleus of the vagus nerve; JG: jugular ganglion; LA: left atrium; LV: left ventricle; NA: nucleus ambiguus; NG: nodose ganglion; RA: right atrium; RCS: right coronary sulcus; RV: right ventricle; –: no labeled neurons.

glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Finally, ducks were perfused with 5% sucrose in 0.1 M phosphate buffer at 4°C. After perfusion, the spinal segments C2 to T6 together with the dorsal root ganglia and paravertebral ganglia, the nodose and jugular ganglia and the brainstem were immediately removed, and these tissues were immersed overnight in phosphate-buffered 30% sucrose at 4°C. Serial frozen sections were cut at 40 μ m in thickness with a cryostat microtome. The sections were processed for HRP histochemistry with benzidine dihydrochloride as chromogen [22] and mounted on glass slides in 2% gelatin and 50% ethanol solution. After drying in air, selected sections were lightly counterstained with 1% neutral red solution.

RESULTS

After injections of HRP into the heart of the duck, retrogradely labeled neurons were bilaterally found in paravertebral ganglia, in the dorsal motor nucleus of the vagus nerve and the nucleus ambiguus of the medulla oblongata, in dorsal root ganglia, and in the nodose and jugular ganglia of the vagus nerve (Table 1). Labeled neurons were not found in any regions of the spinal cord. These findings are described in detail below.

Sympathetic postganglionic neurons: After injections of HRP into the various regions of the heart in the duck, labeled neurons were mainly found in the paravertebral ganglia C16 (the last cervical segment in the duck) to T2 of the

sympathetic trunk. The number of labeled neurons was found to be highest in the paravertebral ganglion T1 (about two-thirds of the labeled neurons), followed by the ganglia T2 and C16 in that order. Only in three experiments, labeled neurons were also observed in the paravertebral ganglion C15 or T3. Following injections into the coronary sulcus or ventricle, the number of labeled neurons was greater than that after injections into the atrium. After injections of HRP into the right side of the heart, some ducks contained more labeled neurons in the right paravertebral ganglia than in the left ganglia and others had more labeled neurons in the left ganglia than in the right ganglia. On the other hand, the ducks injected into the left side of the heart always contained a greater number of labeled neurons in the left paravertebral ganglia than in the right ganglia. Labeled neurons in the paravertebral ganglia were mainly polygonal in shape (Fig. 1). The somal size of labeled neurons was variable but the large majority were small and medium-sized neurons (15–25 μm in diameter).

Vagal preganglionic neurons: In 14 of the 33 ducks, labeled neurons were found in the medulla oblongata of many experiments, especially after injections of HRP into the coronal sulcus. About two-thirds of labeled neurons observed within the medulla oblongata were located in the nucleus ambiguus and the rest were found in the

ventrolateral part of the dorsal motor nucleus. Labeled neurons were observed on the right and left sides of both nuclei between 2.0 mm rostral to the obex and 0.4 mm caudal to the obex, respectively. Labeled neurons in the dorsal motor nucleus of the vagus nerve were spindle in shape (Fig. 2) but labeled neurons in the nucleus ambiguus were polygonal in shape (Fig. 3). The size of the labeled neurons in the nucleus ambiguus (20–30 μm in diameter) was larger than that of the labeled neurons in the dorsal motor nucleus (18–20 μm in diameter).

Sensory neurons: In 14 of the 33 ducks, labeled neurons were found in the dorsal root ganglia C16 to T2 on both sides after injections of HRP into the various regions of the heart. The number of labeled neurons was found to be highest in the dorsal root ganglion T1 (about a half of the labeled neurons), followed by the ganglia T2 and C16 in that order. In only one duck, labeled neurons were also observed in the dorsal root ganglion C15. These labeled neurons were preferentially located at the central part of each ganglion. After injections into the right side of the heart, labeled neurons were found in the right and left dorsal root ganglia. On the other hand, labeled neurons were exclusively detected in the ipsilateral ganglia after injections into the left side of the heart. Labeled neurons in the dorsal root ganglia were round or oval in shape (Fig. 4). More than 70% of labeled neurons

Fig. 1. Labeled sympathetic postganglionic neurons in the right paravertebral ganglion T1 after injection of HRP into the right coronary sulcus of the heart. $\times 125$.

Fig. 2. Labeled parasympathetic preganglionic neurons (arrowhead) in the right dorsal motor nucleus of the vagus nerve after injection of HRP into the right coronary sulcus of the heart. $\times 62.5$.

Fig. 3. Labeled vagal preganglionic neurons in the right nucleus ambiguus after injection of HRP into the right coronary sulcus of the heart. $\times 125$.

Fig. 4. Labeled sensory neurons in the left dorsal root ganglion C16 after injection of HRP into the left ventricle of the heart. $\times 125$.

Fig. 5. Labeled sensory neurons in the right nodose ganglion after injection of HRP into the left ventricle of the heart. $\times 125$.

Fig. 6. Labeled sensory neurons in the left jugular ganglion after injection of HRP into the right atrium and coronary sulcus of the heart. $\times 125$.

were small cells (10–20 μm in diameter).

In 20 of the ducks, labeled neurons were also found in the vagal nodose and/or jugular ganglia. Labeled neurons were observed in the nodose ganglion of many experiments, especially after injections into the right coronal sulcus and/or ventricle. Most of the labeled neurons in the nodose ganglion were ellipsoid in shape (40 μm in length and 20 μm in width) and located in the peripheral zone of the ganglion (Fig. 5). On the other hand, labeled neurons in the jugular ganglion were detected only in the 3 experiments. They were exclusively located in the left ganglion. Labeled neurons were middle-sized cells (20–25 μm in diameter) and round in shape (Fig. 6).

DISCUSSION

Sympathetic postganglionic neurons innervating the heart: In our previous gross-anatomical study, the cardiac sympathetic nerve of the duck was observed to arise from the parasympathetic ganglion T1 or T2, but not from the cervical parasympathetic ganglia [5]. In the present study, however, sympathetic postganglionic neurons innervating the heart were observed in the paravertebral ganglia C15 to T3, especially in the ganglion T1. After transection of the sympathetic cardiac nerve in the pigeon, chromatolytic neurons were observed in the paravertebral ganglia C12 to C14 and the proportion of such cells was highest in the ganglion C14, intermediate in the ganglion C13 and lowest in the ganglion C12 [20]. Moreover, HRP-labeled neurons were found in the paravertebral ganglion C14 following application of HRP to the sympathetic cardiac nerve of the pigeon [3]. Therefore, sympathetic postganglionic neurons innervating the heart of birds are situated in the paravertebral ganglia of the most caudal cervical and most cranial thoracic segments.

In mammals, the location of sympathetic postganglionic neurons innervating the heart are mainly present in the middle cervical and stellate ganglia, but the proportions of postganglionic neurons in these ganglia differ between mammalian species. In the dog, they were located in the greatest number in the middle cervical ganglion and fewer were located in the stellate ganglion [1, 10, 12, 30]. On the other hand, postganglionic neurons innervating the cat heart were predominantly found in the stellate ganglion [18, 19, 23, 26, 32]. In the pig, these neurons were relatively evenly distributed among the stellate and middle cervical ganglia [15]. Although paravertebral ganglia in birds do not fuse to form the middle cervical or stellate ganglion, the difference in craniocaudal distribution of postganglionic neurons innervating the heart was also found between avian species. Postganglionic neurons innervating the duck heart seem to be located in more caudal segments of paravertebral ganglia than those innervating the pigeon heart.

In the present study, the right side of the heart was bilaterally innervated by postganglionic neurons of paravertebral ganglia but the left side of the heart was ipsilateral-dominantly innervated. There was a difference

in sympathetic innervation between the right and left sides of the heart. This may be related to functional differences between the right and left sympathetic cardiac nerves. In the pigeon, electrical stimulation of the right cardiac nerve consistently increases heart rate, whereas this is not true for the left [20]. In parasympathetic and sensory innervation to the heart of the duck, we could not detect obvious laterality.

Parasympathetic preganglionic neurons innervating the heart: Previous studies using electrical mapping methods [7], retrograde degeneration [8] or single unit studies [25] demonstrated that vagal preganglionic neurons innervating the heart of the pigeon were located in the dorsal motor nucleus of the vagus nerve. No evidence of chromatolysis was found in the nucleus ambiguus [8]. Thus, parasympathetic preganglionic neurons innervating the heart of birds were thought to be within the dorsal motor nucleus of the vagus nerve. On the other hand, in a retrograde labeling study using fragment C of tetanus toxin, it was demonstrated that the majority of labeled vagal preganglionic neurons innervating the heart of the pigeon were confined to the nucleus ambiguus and a smaller number of labeled neurons were located in the dorsal motor nucleus of the vagus nerve [4]. In the present study, a quantitative majority of neurons innervating the heart of the duck were located in the nucleus ambiguus and not in the dorsal motor nucleus as previously thought. In mammals, vagal preganglionic neurons innervating parasympathetic postganglionic neurons of the heart have been found in 2 separate regions of the medulla oblongata; the dorsal motor nucleus of the vagus nerve and the nucleus ambiguus [cat: 2, 6, 9, 18, 21, 23, 29; dog: 2, 11; monkey: 14; pig: 15, 16; rat: 24, 27, 28]. A quantitative majority of preganglionic neurons are located in the nucleus ambiguus and ventrolateral nucleus ambiguus. Therefore, it is suggested that the cardiac parasympathetic preganglionic representation in the avian medulla oblongata is very similar to that in mammals.

Sensory neurons innervating the heart: In the present study, sensory neurons were labeled in the dorsal root ganglia C15 to T2 and in the nodose and jugular ganglia. In the pigeon, sensory neurons innervating the heart were found in the dorsal root ganglia C13 and C14 [3], but the presence of nodose and jugular ganglion cells supplying the heart has not been reported in any other species of birds as far as we know. In mammals, sensory neurons innervating the heart were observed in the dorsal root ganglia C8 to T9 [cat: 19], C8 to T6 [cat: 31] or C6 to T6 [dog: 13], and in the nodose ganglion [cat: 18, 23; dog: 13; pig: 15]. These sensory neurons form the afferent limbs of cardiac reflexes, transmitting sensory impulses from the heart associated with the control of circulation.

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