Innervation of Heart in Dog

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(Received for publication, July 22, 1953)

Many histological studies on the innervation of human and animal hearts have been published since Kölliker¹⁾ reported on his study on frog's heart in 1862, leading to many notable observations. Among these studies, the one conducted by Prof. Seto^{2),3)} of this Laboratory on human heart is worthy of special commendation, and his observations and deductions have been fully endorsed by such eminent neurologists as Stöhr^{4),5)} and Hermann⁶⁾. Ábrahám,⁷⁾ in 1940, made a study on the same subject, but the imperfect silver method he had in command debarred him from arriving at the extreme minuteness which Seto's researches could reveal.

Treading in the paths of these illustrious predecessors, I made a similar study with the canine hearts as my objective. I hoped I could contribute something in elucidating the difference of human and canine hearts by a collation of my observations with the results of previous studies. In fact, there were studies on canine hearts including those belonging to partial studies, by Smirnow,^{8),9),10)} Dogiel^{11),12)}, Valedinsky¹³⁾, Cajal¹⁴⁾, Michailow^{15),16),17)}, Worobiew¹⁸⁾ and Lawrentjew¹⁹⁾, but there seems to be many a point of doubtful accuracy in their observations.

My materials were taken from adult canine hearts, with special attention to the atria, and fixed for a long time in 10% neutral formol, which I cut into 40 μ frozen sections and stained with Seto's silver impregnation practiced in this laboratory. The large series of beautiful slides obtained thus I subjected to a close microscopic study.

EXPERIMENTAL

Individual Observations

The Gerlach's²⁰⁾ so-called basal plexus or the Dogiel's²¹⁾ subepicardial plexus, found in the atria and provided with ganglia in places, corresponds to the primary plexus composed of most powerful nerve bundles according to Seto's study on human hearts, being formed at the boundary between the epicardium and the myocardium. This observation holds also by the

canine heart I studied. These primary plexus goes over into the secondary plexus consisting of thinner nerve bundles found in both the epicardium and myocardium, which again changes into the more fine meshed tertiary plexus consisting of still thinner bundles. In the canine heart, the above primary plexus is also formed around the Tawara's node and the His's bundle. Some fine nerve fibres are seen penetrating as far as into the endocardium.

It has been known that the external nerve fibres coming into the primary plexus originate in the vagus and the sympathetic nerves, the former consisting of thin non-medullated parasympathetic fibres and stout medullated sensory fibres. The sympathetic and parasympathetic fibres could be here hardly distinguished histologically.

The distribution of the ganglia in the human heart has been minutely described by Francillon,²¹⁾ and Seto²⁾ confirmed his description. In my canine heart, I found a distribution of ganglia almost identical as that in human heart. So, the majority of the ganglia were found in the dorsal wall of the atria and at the entrance of the venae cavae in the heart, being found, however, sporadically in the other parts of the atria. For example, some nerve cell groups are found in the sulcus interauricularis, septum atriolium, valvula Thebesii, and septum fibrosum atrioventriculare, in particular, in its periphery.

Seto²⁾ succeeded in observing existence of nerve cells also in the secondary plexus in the myocardium of human atria cordis. This observation is very exceptional, according to Hermann⁶⁾, but in my adult canine specimen, frequent aggregations of nerve cells were found in the myocardium, as ascertained by Seto, and Dogiel¹²⁾ and Valedinsky¹³⁾ too report of their discovery of nerve cells in the myocardium of various mammals.

The development of the plexus and ganglia in canine atria is little in arrears in comparison with that in human heart. The large ganglia have generally a connective tissue capsule, while no such capsule is found accompanying the small cell groups (Fig. 1). The ganglia are rarely formed in the nerve bundles, the majority being found around the bundles closely touching them. The external nerve fibres coming in are comparatively small in number. The size of the ganglia is not necessarily proportional to the nerve bundles connected therewith, and often enough, a large ganglion is in contact with a thin nerve bundle.

The microscopic construction of the nerve cells in the canine atria has been described by Smirnow¹¹⁾ and Lawrentjew¹¹⁾, but perhaps due to the imperfection of their staining methods, their illustrations show only a very simple formation of the cells. According to my study, the nerve cells in canine atria are classifiable into the Dogiel's Type I and Type II,

Fig. 1. A small ganglion seen in the pericardium of the left atrium cordis in dog. a small nerve bundle; no existence of connective tissue capsule is seen in the surroundings of the nerve cell group. Seto's silver impregnation, $\times 200$.

as is the case with the nerve cells in human atria (Seto). However, whereas in human hearts the large majority of nerve cells are said to belong to the Type I, in canine hearts, cells of Type II being also present comparatively in a great number.

The principal difference between the nerve cells in canine and human atria consists in that the former are smaller in size and simpler in formation. In canine hearts, the nerve cells are provided with a very ill-developed connective tissue capsule, and even this poor integument is often denied them. The mantlecell-plasmodium is much smaller by canine than by human atria, and consequently the cell nuclei in it are also far fewer in number. The number of the nerve processes emerging from the nerve cells is also fewer than by man, as described hereunder, and the short processes are simpler in their ramification and termination.

The Dogiel's so-called nerve cells Type I are represented by cells with short processes terminating in the mantlecell-plasmodium. The long processes thereof are mostly single per cell, but more than two long processes per cell are not rare. The number per cell of the short processes is decidedly smaller than by human atria, and their ramification and terminal formation are much simpler. In Fig. 2, for example, a Type I cell sends out 2 or 3 short processes which terminate after only a very short and simple course in the plasmodium. The Type I cell seen in Fig. 3 has a few more short processes than in the foregoing, but their course and terminal formation are much simpler. In the two examples above, the short processes are seen emerging from any indefinite spot of the entire surface of the cell bodies. Thus, the plasmodium around the cells is nearly equal in thickness in all its parts.

Fig. 2. A most simple nerve cell Type I of Dogiel provided with 2-3 short nerve processes found in the pericardium of the canine atrium. The number of cell nuclei in the mantlecell-plasmodium is very small; b connective tissue capsule. Same staining, $\times 500$.

Fig. 3. A simple nerve cell Type I of Dogiel with several short processes ending in the mantlecell-plasmodium seen in the pericardium of the canine atrium. Cell nuclei in the plasmodium are comparatively numerous. Same staining, $\times 500$.

But the case is different with the cell in Fig. 4. Herein, all the nerve processes emerge from one pole of the cell body, and the short processes end in the plasmodium that is formed here in a cone containing numerous plasmodium-nuclei aggregated in this part. Such Type I cells of specific conformation have been reported by Seto in human atria, but this peculiar sort of cells shows a much simpler formation in my canine heart than that in man.

Dogiel's cells Type II are represented by the cells with short processes running out through the connective tissue capsule to end in the vicinity of the mother cells. In my dog specimen, the nerve cells belonging to the Type II have in general one or two long processes and 3 to 6 short processes. The latter end sharply without undergoing a complex branching, after running out through the connective tissue capsule, as shown in Fig. 5.

Seto²⁾ has observed that the short processes emerging from a Type II

Fig. 4. A specific Type I cell of Dogiel seen in the pericardium of the canine atrium. All the short processes emerge from the one pole of the cell body, to end in the conically formed plasmodium rich in specific cell nuclei. Same staining, $\times 500$.

Fig. 5. A nerve cell Type II of Dogiel found in the canine atrium. All the short processes run out through the connective tissue capsule. Same staining, $\times 500$.

Fig. 6. Ditto. One of the short processes terminates in a conical neuro-fibril end-plate.

cell in the human atria often end in bulb-like or oval terminal plates, and some of them end buried in another nerve cell body. Moreover, such

end formations are often found originating in plural number from one single cell. In my specimen of canine atria, such formation of terminal plates on the short processes from nerve cells, especially from cells Type II, was found to be extremely rarer than in the case of human heart. Fig. 6 shows one of such rare examples, where only one end plate is formed at the termination of one of the short processes sent out from a cell Type II.

The observation by Stöhr²²⁻²⁵⁾, Reiser^{26), 72)} and Seto²⁾ revealing the forming of netlike ending surrounding the outside of the plasmodium of the nerve cells in common, called pericellular terminalreticulum, by the incoming vegetative fibres reaching the periphery of the cells in the vegetative nerve ganglia, upon branching out into very fine fibres, has been amply confirmed also by my study on the ganglia in the canine atria. However, in canine heart, the pericellular terminalreticulum is rarely formed by so colossal a number of minute fibres as by man, but in majority of cases, is formed by a more dispersed interlacing arrangement of somewhat thicker fibres. Thus, it was confirmed that this terminal formation is also much simpler in the canine heart than in the human heart.

Up to some 20 years ago, the termination of the vegetative nerve fibres was deemed to consist of free endings, much as the endings of sensory and motor nerve fibres. But through the laborious researches by Stöhr, Reiser and Seto, it has been established that their ending is represented by the so-called vegetative terminalreticulum arranged in the form of spread-out net. Many later researchers confirmed this proposition, and the fact has been now verified beyond any dispute. This holds true also of the termination of the vegetative fibres in the heart, both sympathetic and parasympathetic. Formerly, Smirnow¹⁰⁾, Michailow¹⁷⁾, Lawrentjew¹⁸⁾ and Boeke²³⁾ reported that both the sympathetic and parasympathetic nerve fibres had separate and independent free endings, but Seto²⁾ was the first in establishing the existence of vegetative terminal-reticulum common to both kinds of vegetative fibres in his study on human heart in 1936. This discovery of terminalreticulum in the human heart was highly lauded by Stöhr^{24), 25)}, Hermann⁶⁾ and other investigators, who confirmed the existence of such reticulum. In 1940, Ábrahám⁷⁾ confessed his inability to affirm the observation of Seto, but I fear this was due to the imperfection of staining method he adopted, which caused his failure in properly staining the most delicate nerve fibrils.

In my canine specimen too, the sympathetic and parasympathetic nerve fibres, accompanying the long processes originating in the nerve cells in the atria, run out of the plexus peripherally, the bundles of these nerve fibres gradually decreasing in size, forming anastomosis with other nerve bundles at frequent intervals, and further decreasing in size. Then,

the nerve fibres go over into the Reiser's preterminal fibres, finally to end in the terminalreticulum.

The terminalreticulum is represented by the nerve cord in which the minutest neuro-fibrils which cannot be divided further, spread out reticularly. The terminalreticulum contains Schwann's nuclei in places, and stands in tactile control over almost any kind of tissue cells.

The terminalreticulum is found in a large number in all the layers of the atria. In the epicardium, it is spread out in the connective tissue and fat tissue, and in the myocardium, along the heart muscle fibres. In the latter part, it runs also along the blood vessels, especially, the capillaries, standing in a common control relation thereto, and never ends freely. In a similar manner, the terminalreticulum in the heart always acts in common supply over the controlled cells.

The terminalreticulum is formed most abundantly and in a most typical formation in the walls of the arteries. The terminalreticulum running among the heart muscle fibres is also comparatively numerous, showing the common supply of capillaries here, too (Fig. 7). In this case, the terminalreticulum comes in contact with the tissue cells but does not penetrate into the cell bodies.

Fig. 7. Vegetative terminalreticulum (t) in the myocardium of the canine atrium. s sensory nerve fibre running with vegetative preterminal fibres of Reiser. Same staining, $\times 800$.

As for the sensory nerve endings found in the hearts of animals, Smirnow⁸⁾ was the first in describing rather complex branched terminations in the amphibian heart and complex arborized terminations in the epicardium of rats and cats in 1895, which observations were confirmed by Dogiel¹¹⁾. Michailow^{15), 16)}, in 1907/08 described the existence of capsulated and uncapsulated branched terminations in the endocardium and epicardium in horse. However, as the above observations were made in specimens stained with methylene blue, there seems to be some justification for further scrutiny to determine their accuracy. Lawrentjew¹⁹⁾ (1929) observed

complex arborized endings in his study on the hearts of dogs and cats with silver method. Lawrentjew and Gurwitch-Lasowskaya²⁹⁾ (1930) reported their discovery of a certain type of sensory nerve endings in the connective tissue in the atrioventricular bundle in rat. As may be readily surmised from the above enumeration, the study on the sensory nerve terminations in the hearts of animals may be termed as yet very sporadic.

On the other hand, in the case of human hearts, there is the thoroughly dependable research by Seto²⁾, which brought many detailed observations on the terminal formations of the sensory fibres to light. I also succeeded in observing numerous sensory terminations of various types in my very beautifully stained preparations of canine heart and in collation with the findings of studies on human heart (Seto), have arrived at the following results.

Somewhat against my expectations, I found many thick medullated nerve fibres in the heart of dog. These I take to be sensory fibres derived from the vagus in their majority, as Seto describes such fibres in the human heart. These fibres all terminate freely, and their terminations can be divided into the unbranched, the simple branched terminations with 2 or 3 rami and the terminations of Type I connected with the falling reflex of blood pressure formed rather smaller in size than in human heart. But I could not find those extremely complex branched endings described by Smirnow⁸⁾ and Dogiel¹¹⁾, nor the glomerular endings reported by Michailow^{15), 16)} in horse's heart. Thus, there are rather numerous sensory terminations found in canine heart, but in construction, these are far simpler than those found in human heart. There are also some differences between canine and human hearts in the distribution of sensory terminations.

In addition to the above, I could discover many a sensory termination penetrating into the externa and sometimes the media of the branches of aa. coronariae running through the epicardium. Some of these terminations even showed considerable complexity in their formation.

Now, the unbranched terminations are the simplest of the sensory endings. A stout medullated fibre, after losing its myelin, runs the winding course peculiar to sensory fibres, showing some change in size, generally to end sharply. Such terminations are found in any of the layers of the atria, with special predominance in the myocardium.

Fig. 8 shows a typical unbranched termination found in the myocardium of a dog. In some cases, these terminations show a characteristic ansiform course, that is, a very simple glomerular formation, as shown in Fig. 9. Unbranched terminations are found often in the epicardium as well, attention being specially invited to the existence of some of them running in close contact with the basis of the endothelial cell layer of the epicardium. Often some of the unbranched terminations found in the

Fig. 8. A typical unbranched sensory termination found in the myocardium of canine atrium cordis. Same staining, $\times 200$.

Fig. 9. An unbranched sensory termination showing a simple ansiform course found in the myocardium of the canine atrium. Same staining, $\times 800$.

endocardium are observed as formed by stout sensory fibres meandering in close contact with endothelial cells, as shown in Fig. 10.

The nature and running course of nerve branches in the simple

Fig. 10 An unbranched termination represented by a thick sensory nerve fibre and ending direct under the endothelial cell layer of the endocardium. Canine atrium cordis. Same staining, $\times 500$.

branched terminations are similar to those in the unbranched terminations, except that in some cases, the branches end not sharply but bluntly, and sometimes also forming terminal bodies formed by fibril dissolution. These also may describe ansiform course and they are found everywhere, in the endocardium, in the myocardium as well as in the epicardium, especially abundant in the myocardium but in a small number in the endocardium.

Fig. 11 shows a simple branched ending found in the connective tissue at the boundary of the pericardium and the myocardium. The 2 or 3 rami, after a peculiar winding course, end bluntly or upon completing an ansiform course. Fig. 12 shows a simple branched termination found in the myocardium, with the rami showing a rather remarkable change in

Fig. 11. A simple branched ending found at the boundary between the pericardium and myocardium of the atrium cordis dextrum in dog. Terminal branches end sharply or bluntly. Same staining, $\times 500$.

Fig. 12. A simple branched sensory termination found in the myocardium of the atrium cordis in dog. Same staining, $\times 320$.

size. Fig. 13 shows another simple branched termination, showing formation of terminal bodies in several parts. This termination is formed by a thick sensory fibre in its course of running together with a few other sensory and vegetative fibres.

The sensory terminations Type I connected with the depressor reflex, discovered by Sunder-Plassmann³⁰⁾ and de Gastro³¹⁾ in the carotic sinus, have been found by Seto²⁾ in human arcus aortae as well as in the human

Fig. 13. A simple branched sensory termination formed in the running course with the other nerve fibres, having special neurofibril bodies. Myocardium of the left atrium in dog. Same staining, $\times 500$.

atria cordis³⁾, in particular, in the connective tissue of the myocardium. I could also observe such terminations, quite similar to the human counterparts in formation, in the canine endocardium, especially in the endocardium adjacent to the limbus fossae ovalis.

These endings are formed, as shown in Figs. 14 and 15, by sensory

Fig. 14. Termination Type I connected with the falling reflex of blood pressure seen in the endocardium in the surroundings of fossa ovalis of the canine atrium cordis. Many special end-plates are seen along the terminal branches and in their tips. Same staining, $\times 800$.

fibres with very stout trunk fibres, which branch out in numerous rami changing in size after branching and represented as strong fibres. Sometimes in their running course, but in most cases at the tip of the terminal branches there are formed very peculiar terminal plates due to fibril dissolution. These endings are accompanied by numerous globular special nuclei.

In human heart, some considerably complex branched sensory terminations have been found around the small veins in the myocardium (Seto)²⁾ but in my canine heart, sensory terminations were extremely rare around the veins. However, sensory fibres were found running along the small

Fig. 15. Ditto.

arteries everywhere, which end mostly in comparatively simple branched terminations.

Seto has often found in the human heart extremely complex sensory terminations ending around the heart muscle fibres, but in my canine specimen, I found no such complex terminations related to the muscle fibres, although very simple sensory terminations were not rarely found around them.

SUMMARY

In canine, as in human, atria cordis, the primary plexus is formed at the boundary between the epicardium and myocardium, which goes over into the secondary and tertiary plexus formed in the epicardium and myocardium. Minute fibres are also observed running into the endocardium.

The incoming nerve fibres running into the primary plexus originate in the vagus and sympathetic nerves, the former consisting of non-medullated fibres, so that the distinction between sympathetic and parasympathetic fibres is not possible histologically.

The distribution of nerve cell groups found in the plexus in the epicardium is also similar by human and canine hearts. Ganglia are mainly found in the dorsal walls of the atria and the entrance of the venae cavae into the heart, but sporadically in all other parts of the atria. In canine heart, as in human (Seto), nerve cell groups are found also in the secondary plexus in the myocardium.

Nerve cells in the canine atria can be also classified into the cells of Dogiel's Type I and Type II, of which the latter exist somewhat more abundantly by dog than by man. The nerve cells in canine atria are much smaller in size and simpler in construction than those in human heart, e.g., the pericellular connective tissue capsule being poorly developed, often

even to total disappearance, the mantlecell-plasmodium being limited in size and the plasmodium-nuclei very much poorer in number. The number of nerve processes is also smaller than in human heart, especially with the nerve cells Type I, and the ramification and terminal formation of the short processes are much simpler.

The plasmodium surrounding the nerve cells Type I is generally aligned in a uniform thickness, but as is the case in human heart, frequently the plasmodium is found especially developed at one pole of the cell. But the arrangement of short processes branching and ending therein is far simpler than in man. In nerve cells Type II, the number of short processes is rather smaller than in man, and the terminations thereof more rarely end in special plates.

The terminations of incoming nerve fibres approaching the intragangliar nerve cells are represented by pericellular terminalreticulum, as in man, but these are much simpler than in man in construction. In dog, rather thick fibres are arranged in a comparatively dispersed interlacing mesh.

The termination of vegetative nerve fibres is represented by the terminalreticulum in canine atria as in human atria. This comprises the three kinds of elements derived from the incoming sympathetic and parasympathetic fibres and the long processes from the nerve cells, consists of nerve cords formed by extremely minute fibrils arranged reticularly, ends never freely, and stands in tactile supply over any of the controlled cells.

A considerable number of medullated sensory fibres is seen running in canine atria. These end freely, and in form, can be classified into the unbranched endings, the simple branched endings and the endings Type I connected with the depressor reflex. These endings, however, are always much simpler in formation than in man. Their localization is also somewhat different from that in man. Beside the above kinds of terminations, there are found branched sensory endings in the walls of the branches of aa. coronariae cordis.

The unbranched terminations are found in the epicardium, the myocardium and the endocardium of the atria, but in special abundance in the myocardium. They end mainly sharply, but sometimes also upon completing an ansiform course. It is noteworthy that such endings are often formed close beneath the endothelial cell layers of the epi- and endocardium.

In the simple branched endings consisting of 2 or 3 rami, the branches end generally sharply, but some end bluntly or either in a terminal body formed by fibril dissolution or describing a simple ansiform course. These endings are found in all the layers of the atria, but are particularly abundant in the myocardium and few in the endocardium.

The sensory terminations Type I connected with the depressor reflex are also found in canine atria, particularly in the endocardium around the fossa ovalis. They are poorer in scale by dog than by man (Seto), but the nature of the trunk fibres and the rami and the formation of special terminal plates through fibril dissolution are nothing different from those in man.

Unlike the case in human heart, sensory endings are rarely formed around the small veins, and if any, their terminations are very simple. But the number of sensory fibres running along the small arteries is very large, all ending in simple branched terminations. No complex sensory terminations are found surrounding muscle fibres in the canine atria.

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