THE MYOGENIC AUTOMATISM OF THE SYSTEMIC HEART OF OCTOPUS VULGARIS (CEPHALOPODA: COLEOIDEA): EVIDENCE FOR LOCALIZED STRETCH SENSITIVITY AND PACING ACTIVITY

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ABSTRACT: The localization of the stretch sensitivity and myogenic automatism in the systemic heart of Octopus vulgaris has been studied on an isolated preparation in which the ventricle was zoned by ligatures. Each region has been submitted to two different levels of internal hydrostatic pressure (1 and 2 kPa). Only the two atrio-ventricular regions were able to contract regularly when submitted to internal pressure, with a frequency dependent from the pressure value, while the ventricle-aortic region was insensitive to the stretching by internal pressure. This result supports the hypothesis that the automatism in this heart is localized. Electrocardiogram recordings from different areas of an isolated and perfused preparation of the systemic heart ventricle are also reported, which suggest that the electrical activity of the ventricle originates in two narrow areas near the atrio-ventricular valves.

KEY WORDS: Octopus - systemic heart - automatism - stretch.

INTRODUCTION

Cephalopods are active organisms with high metabolic rates (O’Dor and Webber, 1986), so that they must have a fast and relatively efficient blood supply system. Cephalopods have developed a closed circulatory system characterized by a high systemic pressure and a powerful single central heart, the systemic heart, situated on the oxygenated side of gills. This central pump consists of a single ventricle and two auricles (left and right). It has a relevant myocardial mass, comparable with that of fishes, (Agnisola and Houlihan, 1994) and a complex myoarchitecture (Kling and Schipp, 1987). A lacunary-vascular system for blood supply to the ventricle has been described (Agnisola et al., 1990). This system connects the intraventricular lumen with superficial epicardial veins through a capillary bed.

The systemic heart of cephalopods is characterized by a myogenic autorhythm which is under neurohumoral control (Kling and Jakobs, 1987; Jakobs and Schipp, 1992). The isolated octopod ventricle contracts in a regular way for many hours if stretched by internal hydrostatic pressure; heart rate and contractile force are related to the input pressure (Smith, 1981a; Foti et al. 1985; Agnisola and Houlihan, 1991). The need for an internal pressure to get the regular beating of the isolated systemic heart suggests that stretch sensitivity and automatism in this heart are intimately related. The hearts of Octopus and Eledone (Smith, 1981b) as well as Sepia (Jakobs and Schipp, 1992) display well defined electrocardiogram (ECG) waves, and the existence of a precise sequence for the depolarization of the ventricular myocardial wall has been proposed (Smith, 1981b). However, it is still unknown whether there is in this heart a distinct pacemaker region, and an associated localized stretch sensitivity, or whether each myocardial cell possesses the character of pacemaker activity (diffuse myogenic automatism).
In the present paper the effect of wall stretching by internal pressure on different regions of the ventricle has been studied by zoning it with ligatures. This approach allows to test if the stretch dependency of heart activity is diffuse in the ventricle or is limited to specific regions of its wall. Moreover, a first attempt to make a surface map of unipolar ECG records of the isolated systemic heart aimed to obtain insights on the impulse propagation on the ventricle surface is reported.

MATERIALS AND METHODS

ANIMALS

The study was carried out at the Zoology Station of Naples "A. Dohrn", Naples, Italy. Six specimens of either sex of Octopus vulgaris (0.5-1.5 Kg in weight) were captured in the Bay of Naples and maintained in circulating seawater pools for at least one week before use.

HEART PREPARATION FOR TESTING REGIONAL STRETCHING SENSITIVITY

The animals were sacrificed by decapitation and the systemic heart dissected out. The dissection was made at 4°C. The isolated ventricle was zoned in three regions of similar size by ligatures, as shown in Fig.1. Dorsal aorta and both the auricles were cannulated, while gonadal and abdominal aorta were ligatured at their bases. The dorsal aorta was cannulated forcing the aortic valve.

In each preparation the cannulae were alternatively connected to an input reservoir to give a fixed head pressure. In this way each region was separately subjected to internal hydrostatic pressure and its mechanical activity was estimated by an isotonic transducer (UGO BASILE Biol. Res. Apparatus) connected to a chart recorder. Two different pressure levels were used (1 and 2 kPa). The perfusion saline was filtered seawater containing 0.05% glucose, pH 8.0, gassed with oxygen. Because of the ligatures, there was no flow through the heart, apart some outflow from the coronary veins. Measures were made at room temperature.

ISOLATED SYSTEMIC HEART PREPARATION AND ECG DETERMINATION

The isolated systemic heart was prepared according to Agnisola et al. (1989) with some modifications. The dissection was made at 4°C. Both the auricles and the dorsal aorta were cannulated, while gonadal and abdominal aorta were ligatured at their bases. The perfusion apparatus was as reported by Agnisola et al. (1989). The perfusion saline was same as described above. The perfusate was not recirculating and the two auricles received it at the same controlled input pressure. The basal perfusion conditions were chosen to reproduce in vivo resting afterload and stroke volume values as previously reported (Agnisola et al. 1989; Agnisola et al., 1994). Each heart was generating its own rhythm. Preparations were stable for at least one hour.

For the unipolar recording of the ECG preparations were enclosed in a Faraday-cage. Two platinum electrodes (0.2 mm in diameter) were used. One of the electrodes was grounded while the other was inserted into the ventricle surface where necessary. The signals were amplified and recorded with a dual beam oscilloscope (502A, Tektronic Inc.). The measures were made at room temperature.
RESULTS

STRETCH DEPENDENCE OF DIFFERENT REGIONS OF THE VENTRICLE

In order to test the stretch dependence of different regions of the \textit{Octopus} ventricle, this has been divided into three regions by two ligatures (Fig. 1): the left and right atrio-ventricular (A-V) regions (indicated as I and II respectively) and the ventricle-aortic (V-A) region (indicated as III). Each cavity in which the ventricular lumen was divided by the ligatures was separately submitted to two different levels of internal pressure (1 and 2 kPa). Two have been the main results of these experiments. First, only the regions I and II respond to the internal load with a regular beating. No spontaneous contraction was observed in the region III when subjected to internal...
pressure independently from the other regions. Second, in the regions I and II both frequency and amplitude of contractions increased with increasing pressure. In particular the doubling of pressure almost doubles the heart rate (from 15± to 27.6±6 beats min\(^{-1}\) in the region I, from 18±4 to 40.5±8 beats in the region II; mean of three preparations). The differences between the left and the right sides were not significant. Relatively high pressures were necessary to obtain contraction rates similar to those of the whole heart perfused preparation or in vivo (Agnisola and Houlihan, 1991; Agnisola et al., 1994).

In summary the above results seem to demonstrate that the stretch sensitivity and the pace control in the Octopus systemic ventricle is localized and not diffuse. In this respect the two atrio-ventricular regions seems to be qualitatively equivalent.

**ELECTROCARDIOGRAM IN THE ISOLATED AND PERFUSED VENTRICLE**

As shown in Fig.2, the isolated ventricle perfused under basal conditions simulating the resting physiological ones (Agnisola et al. 1989) displays a regular and complex ECG waveform. According to previous reports (Smith, 1981b; Jakobs and Schipp, 1992; Agnisola and Houlihan, 1994), a regular R wave, indicating the ventricular depolarization, can be observed. In some preparations a small and not constant P wave occurs (auricle depolarization, Jakobs and Schipp, 1992). Auricles do not contract regularly. The R spike is of 300-500V and it is known to precede the ventricle contraction (Agnisola and Houlihan, 1994).

Interestingly, the shape of the R wave depends on the site of recording. In general, this wave consists of a positive-negative deflection whose relative amplitude depends on the specific region of the ventricle from which it is recorded. An interesting result is shown in Fig.3, where the records obtained from different places of the ventral surface of the isolated perfused ventricle are reported. It can be seen that there are two limited areas, close to each atrio-ventricular junction, in which, unlike the complex waveform detectable elsewhere, only a negative wave is obtained. The absence of a positive deflection indicates these areas as sites of origin of electrical activity in the ventricle.

**DISCUSSION**

Based on the presence of a "P"-wave in the ECG of the Sepia systemic heart, Jakobs and Schipp (1992) have hypothesized the existence of a non-diffuse automatism within this heart and of a specific pacemaker function in the auricle. However, this does not solve the question of whether the ventricle possesses a localized and non-diffuse pacemaker capacity. In the isolated working heart preparations here used the auricles scarcely contribute to the heart rhythm. Their beating is irregular and not synchronous with the ventricular beating. This suggests that the ventricle must have its own pacemaker capacity.

The main result reported here is the apparent regional sensitivity to pressure changes in the systemic ventricle of the octopod O. vulgaris. The zonation of the ventricle by ligatures has clearly demonstrated that the ventricular-aortic region is insensitive to the wall stretching by internal pressure and is unable of spontaneous activity. On the other hand both the atrio-ventricular regions are able to contract
Fig. 2. Typical unipolar electrocardiogram recorded from the isolated and perfused Octopus systemic heart.

Fig. 3. R-waves recorded from different sites of the ventral surface of the isolated and perfused Octopus systemic heart. The determinations were repeated from the same points on three different preparations: no qualitative differences in the R-wave shape were observed.
regularly when submitted to a pressure load. Interestingly, heart rate in these regions seems to be related to the internal pressure. These results strongly support the number of previously reported indications of a ventricular myogenic automatism localized in the vicinity of A-V valves. A "particular sensitivity" in the atrio-ventricular junctions in the Octopus heart has been reported (Skramlik, 1941) and spontaneous contractions within this area in unperfused heart preparations of octopods (Smith, 1981b) have been observed. Some authors have reported that the A-V areas seem to be the origin of the ventricular contractions both in vivo and in vitro (Wells, 1979; 1983). In other molluscs (e.g. Dolabella auricularia, Kuwasawa, 1979) the myocardium within the A-V valve possesses both the ultrastructural and electrophysiological characteristics of a pacemaker.

The conclusion of a localized pacemaker activity in the Octopus ventricle is also supported by the unipolar ECG records in the whole isolated and perfused heart preparations. The analysis of the R-wave shape in different points of the ventricular surface indicates that there is a precise sequence of depolarization of the ventricle, according to the hypothesis of a non-diffuse origin of the activity, and that the probable sites for a localized ventricular pacemaker are the A-V junctions.

The results reported here do not allow to identify specific nodal areas for which a combination of both, histological and electrophysiological studies are necessary (Jones, 1983). However, they are consistent with a non-diffuse automatism in this heart.

REFERENCES

Agnisola: Octopus heart automatism


(Received: 13 January 1994)