Monoamine-containing cells in atrioventricular valves of the opossum heart

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Summary. Using a specific technique for biogenic amines, similar cells to those described as small intense fluorescent (SIF) cells were identified in the atrioventricular valves of the opossum heart. It is suggested that these cells, under neural control, may secrete amines.

Although several workers have shown that the atrioventricular valves contain adrenergic nerves, the function of these elements is still not understood[1-3]. Lipp and Rodin[4] hypothezized that they might secrete norepinephrine into circulation, and according to Anderson[5], they might serve an afferent function. Contemporary studies of adrenergic innervation were facilitated by the introduction by Falck[6] of a specific technique for biogenic amines. Using this technique, various studies of heart innervation of different animal species were done[7,9].

The comparative approach to the adrenergic innervation is useful, since many problems can be better understood, and because a knowledge of the patterns of innervation may give new insight into the mechanisms operating in mammals and in man also[10].

The present study deals with the adrenergic innervation of the atrioventricular valves of the opossum (Didelphis azarae, Temminck 1825), in the hope of gaining a better understanding of valvar function and providing basis a for new interpretations of the production of biogenic amines by the heart.

Material and methods. We studied 4 adult opossums weighing 1.0–2.5 kg. They were killed by exsanguination under ether anesthesia. The hearts were quickly removed, and as soon as the ventricular chambers had been opened, the atrioventricular valves were dissected under the stereomicroscope. To obtain consistent and duplicable results, the heart valves must be excised 10–12 min after sacrifice.

In accordance with Falck-Hillarp's method, the excised valves were rinsed in cold calcium-free Tyrode solution for 2 min, stretched and mounted whole on glass slides and allowed to dry in air at room temperature for 15 min. The relative humidity of the air was maintained at approximately 50% which proved satisfactory for drying the valves, and did not produce any changes in the morphology of the adrenergic nerves as revealed by fluorescence method.

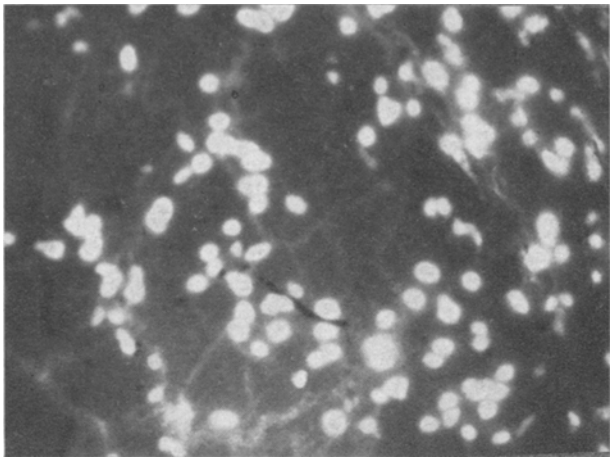

Fig. 1. Tricuspid valve, whole mounted-stretch preparation. Fluorescence micrograph × 250.

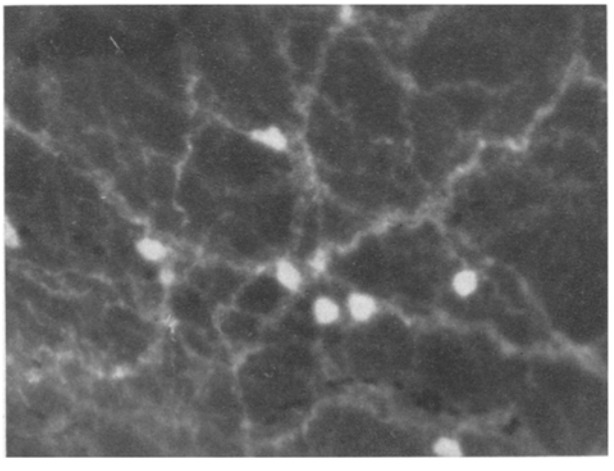

Fig. 2. Tricuspid valve, whole-mounted stretch preparation. Fluorescence micrograph × 400.

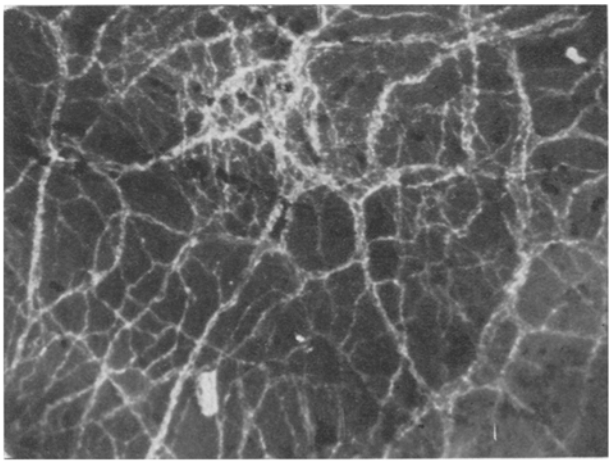

Fig. 3. Mitral valve, whole-mounted. Adrenergic varicose nerve endings forming a dense network. Fluorescence micrograph × 250.

1 T. L. Cooper, L. M. Napolitano, M. J. T. Fitzgerald, K. E. Moore, W. M. Daggett, V. L. Willman, E. H. Sonnenblick and C. R. Hanlon, Archs Surg. *93*, 767 (1966).
2 B. Ehinger, B. Falck and U. Stenevi, Experientia *25*, 742 (1969).
3 A. L. Ferreira and A. M. Rossi, Acta Anat. *87*, 57 (1974).
4 W. Lipp and M. Rodin, Acta Anat. *69*, 313 (1968).
5 R. H. Anderson, Experientia *27*, 1064 (1971).
6 B. Falck, Acta physiol. scand. *56* (suppl. 197), 1 (1962).
7 A. Dahlström, K. Fuxe, E. Mya-Tu and B. F. Zetterstrum, J. Physiol. *209*, 690 (1965).
8 B. Ehinger, B. Falck, H. Persson and B. Sporrong, Histochimie *16*, 197 (1968).
9 D. Jacobowits, Pharmac. exp. Ther. *158*, 1227 (1967).
10 G. Burnstock, Pharmac. Rev. *21*, 247 (1969).

The preparations were then placed in Coplin jars containing paraformaldehyde powder equilibrated with air at 50% relative humidity. The jars were sealed and the preparations exposed to formaldehyde gas at 80 °C for 1 h. Prior to inspections under the fluorescence microscope, the preparations were mounted with non-fluorescent immersion oil.

Results and discussion. Small fluorescent cells were made visible along the whole mounted stretch preparations of the atrioventricular valves of the opossum, prepared according to the Falck-Hillarp method for biogenic amines (figure 1).

These cells were very numerous and exhibit intense yellow-green fluorescence. The nuclei were not visible, and groups of cells were invariably permeated by the dense networks of adrenergic nerves (figure 2). The endings are varicose and similar to those described by others investigators[4,8,11] (figure 3).

Several workers[7–9,12] have used the same technique to visualize monoamine-containing structures in the mammalian heart, and some have reported the presence of fluorescent cells in the atria.

Although it is agreed that the fluorescent reaction product in these cells indicates the presence of a biogenic amine, the precise nature of this substance is controvertial. The study of amine containing cells in several species revealed that they might participate in modulation of ganglionic transmission, since they are in the vicinity of intracardiac parasympathetic ganglia[9]. Ehinger et al.[8] found no evidence of any functional relationship between these cells and the intracardiac ganglia. They are disposed in different topographical localizations within the heart, and it has been demonstrated in different animals that during the phylogeny these cardiac amine-containing cells were more concentrated in the atria. They are thought to function in an adrenergic excitatory control of the heart, at least in lower forms[13]. Using the same histochemical technique, amine-containing cells also have been demonstrated in mammals and in human fetal heart[8,9,13,14]. The literature contains no definitive account of functions of the mammalian amine-containing cells. The cells observed by us showed the characteristics of the small intensely fluorescent (SIF) cells similar to those first described in the sympathetic ganglia[15,16]. These numerous cells suggest humoral rather than neural adrenergic control of the opossum heart.

The well developed neural apparatus and the amine-containing cells found in cusps of atrioventricular valves of the opossum would suggest that, under neural control, both mitral and tricuspid valves are areas representing zones for amine liberation from these cells that might directly affect the receptors localizated in carotic sinus, and a possible mechanism of discharge of afferent stimulations.

11 A. L. Ferreira, J. C. M. Santos and A. M. Rossi, Experientia *31*, 82 (1975).
12 E. M. Krokhina, Acta Anat. *74*, 214 (1969).
13 B. J. Gannon and G. D. Cambell, Z. Zellforsch. *131*, 437 (1972).
14 E. T. Angelakos, Ann. N. Y. Acad. Sci. *156*, 219 (1969).
15 S. Partanen and O. Korkala, Experientia *30*, 708 (1971).
16 O. Eränko, L. Eränko, Prog. Brain Res. *34*, 39 (1971).

Action of alkylating agents on nitrogen fixation by clones of Anabaena doliolum Bharadwaja

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Summary. The physiological and mutagenic actions of three alkylating agents have been studied on the process of nitrogen fixation by *Anabaena doliolum* Bharadwaja. The results show that these chemicals presumably switch off the activity of nitrogenase, resulting in the inhibition of fixation.

Few studies[1–6] have been conducted so far on the effects of some alkylating agents on certain blue-green algae. But no reference, except that of Sharma and Kumar[7], is available regarding the physiological and mutagenic actions of these chemicals on the process of nitrogen fixation in a heterocystous species. The present investigation therefore is an attempt in this direction.

The organism chosen was *Anabaena doliolum* Bharadwaja, belonging to the family Nostocaceae of Cyanophyta. Many samples of this species were collected from various rice fields and the clones were raised from spores. Only 2 clones, No. 1 and 4, were selected on the basis of their maximum and minimum nitrogen-fixing rates under identical conditions. 3 well-known and potent mutagenic agents, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), diethyl sulphate (DES) and ethyl methane sulphonate (EMS), were chosen. These have been shown to induce gene mutations, deletions and several types of chromosomal aberrations in various plants[8].

A fresh stock solution of MNNG was prepared in dark by dissolving it in phosphate-citrate buffer of pH 5.4, as the mutagenic activity of this chemical is lost upon exposure to light[9]. The concentration used was 500 µg MNNG/ml. The treatment was given for 1 h at an interval of 20 min each. The treated cells were washed thoroughly with sterilized glass-distilled water. 1 ml suspension of each treated clone was then inoculated into culture tubes, containing 20 ml nitrate-free medium of Allen and Arnon[10]. The tubes were incubated under flourescent light intensity of 1.1×10^4 ergs/cm^2/sec and at a temperature of 28 ± 2 °C. Nitrogen contents of the replicate samples were determined after a growth period of 30 days by the method mentioned earlier[11].

1 H. D. Kumar, Can. J. Bot. *43*, 1523 (1965).
2 H. D. Kumar, Beitr. Biol. Pflanz. *45*, 161 (1968).
3 H. D. Kumar, Arch. Mikrobiol. *63*, 95 (1968).
4 R. S. Gupta and H. D. Kumar, Arch. Mikrobiol. *70*, 313 (1970).
5 R. S. Gupta and H. D. Kumar, Beitr. Biol. Pflanz. *46*, 389 (1970).
6 B. D. Sinha and H. D. Kumar, Ann. Bot. *37*, 673 (1973).
7 V. K. Sharma and H. D. Kumar, Z. allg. Mikrobiol. *13*, 517 (1973).
8 C. Auerbach, Science *158*, 1141 (1967).
9 D. R. McCalla, Ann. Protozool. *14*, 480 (1967).
10 M. B. Allen and D. I. Arnon, Pl. Physiol. *30*, 366 (1955).
11 V. K. Sharma and H. D. Kumar, Experientia *31*, 182 (1975).