# EFFECT OF CATECHOLAMINES ON CYCLIC AMP CONTENT IN AUTONOMIC GANGLIA

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Views on the role of the cyclic nucleotide system in ganglionic transmission have been formed as a result of the study of mechanisms of function of the autonomic neuron. It has been suggested that they trigger certain postsynaptic processes: the slow inhibitory postsynaptic potential through the cyclic AMP (cAMP) system and the slow excitatory postsynaptic potential through cGMP [6, 8]. Cyclic nucleotides transmit effects both of mediators and of modulators of synaptic transmission. Catecholamines can be regarded as belonging to the latter group [10]. Studies of the direction and intensity of changes in the cyclic nucleotide content in ganglia under the influence of catecholamines have been investigated in a number of studies conducted mainly on the cranial cervical sympathetic ganglion of some species of animals [1, 2, 11, 12]. Stimulation of adenylate cyclase by noradrenalin (NA) and dopamine (DA) has been established and correlation has been studied between the intensity of the response to NA or DA and the content of the corresponding monoamine in small, intensely fluorescent ganglion cells [12].

The aim of this investigation was a comparative study of correlations between the basic cAMP levels in a number of autonomic ganglia and to study the effect of NA and DA on the cAMP content in them.

## EXPERIMENTAL METHOD

Male Wistar rats weighing 160-220 g were used. The total number of animals was 34. Experimental and control groups consisted of 8-18 ganglia. Under pentobarbital anesthesia (60 mg/kg) the animals were perfused with cold (4°C) Hanks' solution, pH 7.3. Ganglia of the sympathetic trunk at level L3-L4, the great pelvic ganglion, and inferior ganglion of the vagus nerve were isolated and placed on a cooling table in Hanks' solution (4°C). Intramural ganglia of the heart were dissected under a stereoscopic microscope after exposure of the isolated areas of the atria for 30 sec in 0.02% methylene blue solution in phosphate buffer, pH 7.0, with glucose. After removal of the membranes the ganglia were cut into blocks and incubated for 20 min at 37°C in homogenizers in Hanks' solution with the addition of 30% of Eagle's medium, equilibrated to pH 7.3 by passing through it a gas mixture of 95% $O_2$ and 5% $CO_2$. Theophylline (5 mM) was added to the incubation medium. Control ganglia were incubated a further 12 min in the same medium, and 50 μM of NA or DA was added to the rest for the same time. During incubation the ganglia were prevented from sedimenting by passage of the gas mixture. After the end of incubation the medium was removed and the ganglia quickly homogenized in 5% TCA at 4°C, then centrifuged at 12,000g for 20 min. The supernatant was extracted six times with three volumes of ether, saturated with bidistilled water, and dried under a jet of nitrogen. The protein concentration in the residue was determined by a micromethod with Coomassie brilliant blue [9]. The cAMP content in the samples was measured with a kit of reagents from Amersham Corporation (England) in the version appropriate for determination of 0.05-4 picomoles cAMP. The results were expressed relative to the protein content in the ganglion.

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TABLE 1. Relative Basic Levels of cAMP and Its Changes under the Influence of Catecholamines in Autonomic Ganglia (M ± m)

| Ganglia | Basic level, % of that inferior ganglion of vagus nerve | Stimulation, % of initial level | |
|---|---|---|---|
| | | NA, 50 μM | DA, 50 μM |
| Inferior ganglion of vagus nerve | 100 | 92,0±9,5 | 92,2±17,7 |
| Great pelvic ganglion | 107,5 | 287,3±43,1 | 123,0±44,5 |
| Lumbar ganglia of sympathetic trunk | 123 | 323,2±13,4 | 164,5±33,7 |
| Intramural ganglia of heart | 82,5 | 165,0±40,7 | 225,4±47,9 |

## EXPERIMENTAL RESULTS

Data on the relative content of cAMP in the various ganglia are given in Table 1. The basic cAMP level in picomoles/mg protein in the inferior ganglion of the vagus nerve was taken as 100. The cAMP content in the lumbar sympathetic ganglia and great pelvic ganglion did not differ significantly from 100%. The intramural ganglia of the heart had lower values of the basic cAMP level.

The reaction of the cAMP system to NA and DA was specific for ganglia of each type. Whereas neither mediator caused significant changes in the cAMP content in the inferior ganglion of the vagus nerve, nerve tissue of the remaining ganglia responded to stimulation by catecholamines. A marked increase in the cAMP content under the influence of NA was observed in the great pelvic ganglion and lumbar ganglia, and under the influence of DA in the intramural ganglia of the atria (Table 1). The ratio of the level of stimulation of the cAMP content by NA to that by DA was 2.33 for the great pelvic ganglion, 1.96 for the lumbar ganglia, and 0.73 for the intramural ganglia of the heart.

The impression was thus obtained that receptor systems in different autonomic ganglia are heterogeneous. In the inferior ganglion of the vagus nerve, where the predominant cells are sensory neurons, opportunities for the action of NA and DA are limited. In ganglia in whose function a leading role is played by sympathetic influences, differences may be due both to the modality of the mediator and the principle of organization of ganglionic transmission. The fact will be noted that small, intensely fluorescent cells in the intramural ganglia of the heart contain DA as mediator, whereas in the great pelvic ganglion they contain NA [3, 4, 7]. Ganglia of the sympathetic chain have small, intensely fluorescent cells which may contain both NA and DA [5].

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