ROLE OF THE CYCLIC ADENOSINE MONOPHOSPHATE SYSTEM IN THE EFFECT EXERTED BY SUBSTANCES WITH SELECTIVE INFLUENCE ON MYONEURAL SYNAPSES

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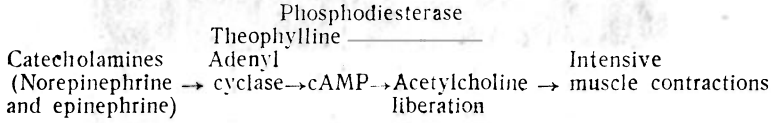
Researches into the role played by catecholamines and by cyclic adenosine monophosphate (cAMP), controlled by them, as a second class mediator in the transmission of nerve impulses within the myoneural synapses have allowed a new interpretation of the well known property of norepinephrine and epinephrine to liberate acetylcholine (ACH) in the mentioned above and in other synaptic formations (8, 10). It has been established that this particular property of catecholamines, similar to many others, is being mediated by cAMP (3, 4). As a matter of fact they stimulate adenyl cyclase which converts ATP into cAMP (9). However, as a rule, cAMP cummulation might be accomplished also by way of phosphodiesterase (PDE) inhibition which cleaves cAMP by hydrolytic route. The xanthine alkaloids theophylline, theobromine and caffeine have been designated as classical or standard (reference) inhibitors of this enzyme. Among them, theophylline, with an inhibition constant equal to 5×10^{-4} M, is distinguished for the highest inhibitory activity. The picture delineating may be schematically presented as follows:



We set out to test this hypothesis (proved to a varying degree) about the role played by the cAMP system in transmitting nerve impulses within the myoneural synapses, and to verify whether or not it is valid also for the action of a number of pharmaceuticals, distinguished for the effect they exert on these synapses, such as D-tubocurarine or activating phosphodiesterases. Imidazole was employed as activator of the latter enzyme in our studies, since it has been proved that it activates PDE when administered at high concentrations (11). On the other hand, the researches of Poletaev (1) show that this substance stimulates acetylcholine liberation.

Material and Method

The experiments were conducted on a series of 18 male cats weighing from 3.5 to 5 kg, under urethane narcosis (2.0/kg body weight subcutaneously — 20 per cent solution 10 ml/kg body weight). The m. gastrocnemius

contractions of the animal experimented upon were recorded upon rhythmic stimulation of the n. ischiadicus motor neuron using rectangular electric impulses with the following parameters: duration 1 msec, frequency 6 impulses/min and current voltage equal to the doubled rheobase. Prior to the beginning of the experiment, the cats were subjected to adrenalectomy with a view to eliminate a rich source of endogenic, hormonal epinephrine, e. g. the adrenal glands.

In most of the experiments the changes in respiration were also recorded.

The substances used were given according to the following scheme: physostigmine — 20 mcg/kg weight — a dose which alone produces no changes in the power of muscle contractions (according to preliminary experiments), but manifests the effects of epinephrine, most likely by way of protecting acetylcholine liberation; epinephrine — 15 mcg/kg weight; caffeine — 10 mcM/kg weight; theobromine — 10 mcM/kg weight; imidiazole — 100, 200 and 300 mg/kg weight; D-tubocurarine — 300 mcg/kg weight. All substances were introduced by intravenous route into the femoral artery.

Results and Discussion

From Fig. 1 it can be seen that physostigmine, epinephrine, theophylline, caffeine and theobromine, given separately at the indicated above doses, do not cause visible changes in the contractions of the cat gastrocne-

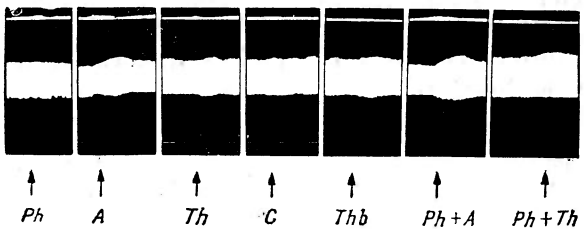


Fig. 1. From top to bottom: respiration; calf muscle contraction after indirect stimulation with rectangular electric impulses;

time interval — 10 sec; Ph — physostigmine 20 mcg/kg; E — epinephrine 15 mcg/g; Th — theophylline 10 mcM/kg; C — caffeine 10 mcM/kg; Thb — theobromine 10 mcM/kg; Ph+E — physostigmine+epinephrine in the indicated doses; Ph+Th — physostigmine+theophylline in the doses indicated above

mius, or else bring about a hardly detectable stimulation (with epinephrine). The combined, simultaneous i. v. introduction of epinephrine (15mcg/kg weight) and theophylline (10 mcM/kg weight) results in a considerable stimulation of the gastrocnemius muscle contractions — averaging 50 per cent in all the experiments. The combined, simultaneous injection of physostigmine and epinephrine accounts for a substantially greater increase of the range of muscle contractions as compared to their independent administration (Fig. 1).

The phosphodiesterase inhibitor theophylline, introduced intravenously, simultaneously with the adenyl cyclase activator epinephrine and physostigmine, leads to a marked stimulation of muscle contraction, much greater than when injected alone (12 per cent) (Fig. 2). The combined injection of physostigmine and epinephrine results in an equally strong musle con-

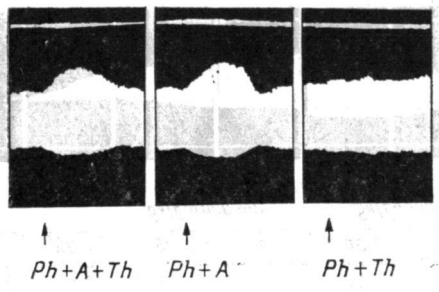


Fig. 2. From top to bottom: respiration; calf muscle contraction during indirect stimulation with rectangular electric impulses;

Ph+E+Th — physostigmine 20 mcg/kg+ +epinephrine+theophylline 10 mcM/kg; Ph+E — physostigmine 20 mcg/kg+epinephrine 15 mcg/kg; Ph+Th — physostigmine 20 mcg/kg+theophylline 10 mcM/kg.

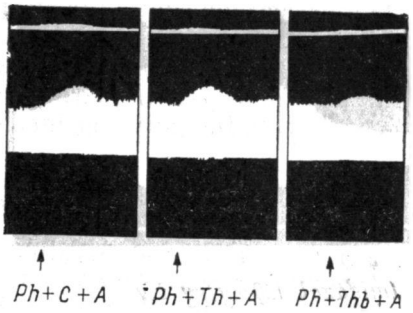


Fig. 3. Top to bottom: respiration; calf muscle contraction during indirect stimulation with rectangular electric impulses;

Ph+C+E — physostigmine 20 mcg/kg+caffeine and sodium benzoate 10 mcM/kg+ +epinephrine 15 mcg/kg; physostigmine 20 mcg/kg+theophylline 10 mcM/kg+epinephrine 15 mcg/kg; physostigmine 20 mcg/kg+theobromine 10 mcM/kg+epinephrine 15 mcg/kg.

traction, whereas when physostigmine and theophylline are given simultaneously, the effect is less pronounced in strength, but lasts for a longer time (Fig. 2).

Upon simultaneous intravenous introduction of physostigmine and epinephrine with the three xanthine alkaloids, administered at equal molar doses, it is seen that the effect of caffeine and theophylline is virtually with equal strength and duration, and is considerably weaker with theobromine (Fig. 3).

The strongest stimulation of the leg muscle contraction is recorded after i. v. imidazole injection at dose 100 mg/kg weight. The mean values of this increase from all the experiments amount to 115 per cent relative to the range of initial contraction (Fig. 4). However, upon imidazole injection at dose exceeding 200 mg/kg weight, a strong inhibition of muscle contraction takes place (Fig. 5).

Epinephrine introduced through endovenous route at dose 30 mcg/kg against the background of a complete neuromuscular block due to D-tubocurarine, promptly restores, almost instantly, neuromuscular conduction to its starting values (Fig. 6).

The results obtained are in support of the hypothesis, already mentioned in the beginning, about the possibility of muscle contractions' intensification by the effect of adenyl cyclase activators (epinephrine) and phospho-

diesterase inhibitors (theophylline, theobromine, caffeine). Although through somewhat different mechanisms, they lead to the same ultimate result — increase of cAMP concentration. This common mechanism unifies in a single group substances, which by chemical structure possess no common features whatsoever, except for their effects being mediated by this second class

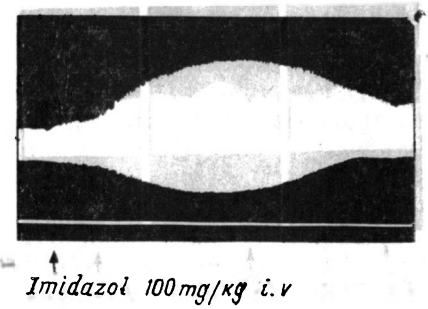


Fig. 4. Leg muscle contraction during indirect stimulation with rectangular electric impulses;
Imidiazole — 100 mg/kg weight.

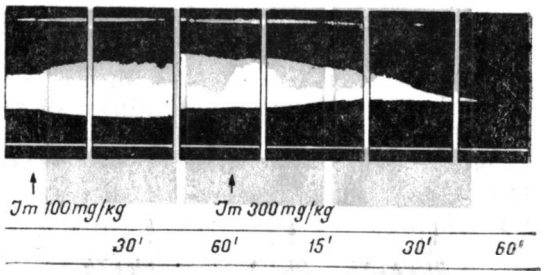


Fig. 5. Top to bottom: respiration; contraction of the calf muscle during indirect stimulation with rectangular electric impulses;
Im — 100 mg/kg weight; Im — 300 mg/kg weight.

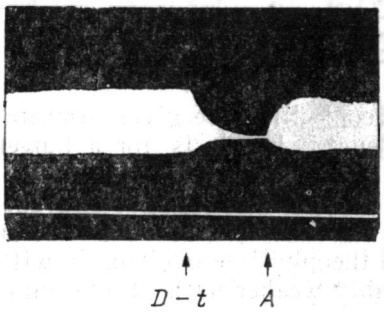


Fig. 6. Contraction of the calf muscle upon indirect stimulation with rectangular electric impulses;
D-t — D-Tubocurarine 300 mcg/kg weight;
E — epinephrine 30 mcg/kg weight.

mediator. In addition, the data of the study performed show that the pharmaceuticals whose chief action is localized in the cAMP system and results in the rise of cAMP concentration, potentiate the contractions of skeletal muscles upon their indirect stimulation. On the other hand, it should be beared in mind that substances as imidazole, for instance, exerting influence on the terminal link in the chain of intermediate processes, connected with muscle contraction, exert effects determined by the terminal linkage proper, and not by the effect they have on earlier stages, especially in case of weaker manifestation of this effect. This is precisely the reason owing to which imidazole, administered at dose 100 mg/kg, intensifies muscle contractions, in all likelihood, on the ground of facilitating acetylcholine liberation (Severin et al, Poletaev) (1, 2). Regarding the blocking of myoneural synapses by elevated imidazole doses (exceeding 200 mg/kg weight), it is quite probably due, on the one hand, to the liberation of blocking acetylcholine quantities, and on the other, to the activation of phosphodiesterase — a phenomenon realized by its higher doses only. In the latter

case, the enhanced enzyme activity of PDE produces a reduction of cAMP, respectively, a decrease of muscle contractions.

Apart from confirming the well known anticurare action of epinephrine, we would like to lay emphasis on the mechanism of its realization through the cAMP system.

In conclusion, it should be stressed that a great deal of caution is required when interrelations are seeked between the effects of various pharmaceutical and other chemical substances and the cAMP system. This is implied by the circumstance that quite often these substances exert effects which, under physiological conditions, are being controlled by the cAMP system, regardless of the fact that they are produced via mechanisms lying out of it.

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О ЗНАЧЕНИИ СИСТЕМЫ ЦИКЛИЧЕСКОГО АДЕНОЗИНМОНОФОСФАТА ДЛЯ ДЕЙСТВИЯ ВЕЩЕСТВ С ИЗБИРАТЕЛЬНЫМ ВЛИЯНИЕМ НА МОНОНЕВРАЛЬНЫЕ СИНАПСЫ

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РЕЗЮМЕ

Изучалась система циклического аденозинмонофосфата в передаче нервных импульсов в мионевральных синапсах на кошках, при помощи активаторов аденилциклазы (адреналина) и ингибиторов фосфодиэстеразы (теофиллина, теобромина и кофеина), а также и имидазола. Установлено, что активаторы аденилциклазы и ингибиторы фосфодиэстеразы приводят к повышению концентрации циклического аденозинмонофосфата, выраженного путем стимуляции сокращений поперечнополосатых мышц при непрямом раздражении. Кроме этого установлено, что имидазол, введенный внутривенно в дозе 100 мг/кг веса, повышает мышечные сокращения на 115% в сравнении с исходными, а в дозе превышающей 200 мг/кг веса — до полной нервно-мышечной блокады.