PARTICIPATION OF MUSCARINIC AND NICOTINIC CHOLINERGIC MECHANISMS IN "KINDLING" FORMATION IN THE CAT AMYGDALA

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The "kindling" phenomenon is widely used at the present time in experiments to study the mechanisms of formation of seizure states and in the search for anticonvulsant drugs. Behavioral effects induced by "kindling" are due to a selective increase in the efficiency of conduction in synapses located at the presynaptic level [13]. An important role in the formation of the "kindling" phenomenon is ascribed to disturbance of the balance between the various mediator systems [6, 10, 14], and also to cholinergic mechanisms, especially muscarinic cholinergic, for direct stimulation of the amygdala by physostigmine, carbachol, and muscarine and its agonists induces epileptiform convulsions in animals, like those observed in response to its electrical stimulation, and their effects are blocked by atropine and scopolamine. Injection of muscarinomimetrics into the limbic structures of the brain also considerably facilitates production of the "kindling" phenomenon by electrical stimulation [11, 12, 15]. It is considered [11] that facilitation of "kindling" is based on an increase in the sensitivity of muscarinic receptors or a decrease in acetylcholine inactivation by acetylcholinesterase (AchE). More recent experimental data have shown that many anticonvulsants, which have proved themselves clinically effective, have a nicotinic cholinelytic action [2].

The aim of this investigation was to study the role not only of muscarinic, but also of nicotinic cholinergic mechanisms in the formation of the "kindling" phenomenon in the cat amygdala.

EXPERIMENTAL METHOD

The "kindling" phenomenon was created in cats with bipolar Nichrome electrodes implanted into the basolateral region of the amygdala under chronic experimental conditions by daily (once or twice a day) electrical stimulation of the amygdala by a current of amplitude (7-10 V, 0.5 msec, 60 pulses/sec, for 15 sec) sufficient to always evoke a major seizure. After 10-13 days the latent periods (LP) of onset of seizures and also threshold values of the current became stabilized. The "kindling" phenomenon was considered to have ended when the animal responded always in a stable manner to the same threshold current of the seizure which usually began with contractions of the jaw muscles (the equivalent of a minor fit), changing into a general clonicotonic fit, which lasted 45-55 sec, and ended in dyspnea, with the animal in a motionless state for up to 2-3 min. The animal then stood up on its limbs and began to respond to the external situation and to the experimenter. Arecoline and nicotine were injected intraperitoneally 4 min, and galanthamine, eterophen, gangleron,* and methyldiazine 15-20 min before the beginning of testing of the "kindling" phenomenon of the amygdala. The numerical results were subjected to statistical analysis by Student's test at the $P = 0.05$ level.

EXPERIMENTAL RESULTS

The muscarinic cholinomimetic arecoline (0.3 mg/kg), the AChE inhibitor galanthamine (1 mg/kg), and the nicotinic cholinolytics eterophen (5 mg/kg) and gangleron (0.5 mg/kg) considerably reduced the duration and intensity of the clonicotonic convulsions (Table 1). Arecoline also reduced the duration of seizures of the jaw muscles. Arecoline, like eterophen, also lengthened the latent period – the time from the beginning of

*1,2-Dimethyl-3-diethylaminopropyl-p-isobutoxygenzoate hydrochloride.

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TABLE 1. Effect of Cholinomimetic and Cholinolytic Drugs and Their Combinations of "Kindling" of the Amygdala in Cats

Drugs	Doses, mg/kg	Temporal characteristics of seizure					
		seizures of jaw muscles, sec	duration of LP until seizure, sec	duration of clonico-tonic seizures, sec	duration clonic seizures	duration of side position after seizures	intensity of seizures
Control	—	63,3±11,7	66,5±10,0	51,2±4,2	—	160,2±42,0c	+++
Arecoline	0,3	26,7±6,0	90,2±6,9	20,2±3,8	—	51,2±18,3sec	+
Eterophen	5	61,7±11,3	90,8±6,5	18,5±5,5	—	51,8±18,7 sec	+
Arecoline + eterophen	0,3+5	—	—	—	—	—	—
Eterophen	10	—	—	—	—		—
Galanthamine	1	60,7±13,7	78,4±20,0	29,7±4,8	—	119,5±4,5sec	+
Galanthamine + eterophen	1+5	18,2±4,5	—	—	—	—	—
Galanthamine	3	67,0±15,0	—	—	—	—	—
Gangleron	0,36	62,5±13,5	62,5±13,5	22,2±5,4	—	120,8±4,3 sec	++
Galanthamine + gangleron	1+0,36	16,5±4,9	—	—	—	—	—
Gangleron	3,5	50,3±13,7	—	—	—	—	—
Galanthamine + gangleron	1+1,5	—	—	—	—	—	—
Nicotine	0,5	53,0±11,8	53,0±11,8	—	62,7±5,8	61,5±6,1 sec	+++
Methyldiazine	0,5	63,3±11,2	66,5±11,0	51,5±5,0	—	157,5±44,9 sec	+++
Nicotine + methyldiazine	0,5±0,5		Seizure at once	80,8±12,0	46,7±6	20,7±3,2 min	+++++
Methyldiazine	1	21,0±5,7	21,0±5,7	92,0±10,8	—	31,2±6,0 min	+
							+++++
Galanthamine + methyldiazine	1+0,5		Seizure at once	123,7±7,0	16,2±3,2	32,0±6,1 min	+++
							+++++

Legend. —) Complete suppression of manifestations of kindling phenomenon, LP) time from beginning of electrical stimulation of amygdala to appearance of clonicotonic convulsions.

stimulation to the onset of clonicotonic convulsions. If arecoline or galanthamine was used in conjunction with eterophen or gangleron in the same doses, all manifestations of the "kindling" phenomenon were completely suppressed. Eterophen in a dose of 10 mg/kg and gangleron in a dose of 3.5 mg/kg also were very effective in this respect.

Nicotine and the muscarinic cholinolytic methyldiazine had quite the opposite action on the kindling phenomenon. Nicotine, in a dose of 0.5 mg/kg, inhibited the tonic, but considerably potentiated the clonic component of the epileptiform response due to the "kindling" phenomenon. Methyldiazine, in a dose of 0.5 mg/kg, has no significant effect on the kindling phenomenon, but if given in the same dose in conjunction with nicotine (0.5 mg/kg) or galanthamine (1 mg/kg), it led to marked potentiation and a considerable increase in the severity of virtually all manifestations of the epileptiform response of this type. Methyldiazine in a dose of 1 mg/kg had a similar effect.

It is an important fact that the seizure in intact animals could not be reproduced again by threshold electrical stimulation until 2-3 h after the first seizure. The use of nicotinic cholinolytics, alone or together with muscarinic cholinomimetics, lengthened this time to 2-3 days. Conversely, after the use of methyldiazine, a seizure could be reproduced whenever the animal stood up on its limbs and began to react to the surrounding situation and to the experimenter.

The results of the present investigation and also data in the literature [9, 14] are evidence that effects of systematic injection of muscarinic cholinomimetics and muscarinic cholinolytics are opposite to the effects of their direct application to the structure concerned. The apparent disagreement between the results of our own experiments and the investigations of Fitz and McNamara [9, 14], on the one hand, and those of Arnold [8] and Albright [7], on the other hand, showing that repeated intraperitoneal injection of atropine in a dose of 25 mg/kg, 1 h before the experiment, delayed the formation of the kindling phenomenon, can be explained on the grounds that, in this case, the process took place against the background of blockade of the ascending reticular activating system (ARAS) by atropine, which impeded the formation of a memory trace, i.e., of a pathological stable state, namely kindling. This view is in agreement with the opinion [13] that seizure activity is based on pathological hyperfunction of the same mechanism as that which is responsible for learning. The present writers, and others [9] used muscarinic cholinolytics and muscarinic cholinomimetics to act on the kindling phenomenon when already formed in the amygdala, when the matrix of this stable pathological state of kindling has already been formed [1] in long-term memory. Under these experimental conditions muscarinic cholinolytics evidently are unable to destabilize and abolish this state, or even to prevent extinction of the kindling phenomenon when formed. It can be tentatively suggested that this is why muscarinic cholinomimetics and, in particular, physostigmine when administered systematically, inhibit the kindling phenomenon in the amygdala.

The absence of synergism and of similarity of direction of the individual effects of physostigmine with atropine, described in [9], and the presence of antagonism alone is evidently due to the fact that, as Prozorskii [5] observed, physostigmine has not only an anticholinesterase action, but also a muscarinic cholinosensitizing and muscarinic cholinomimetic action, whereas galanthamine characteristically has muscarinic cholinomimetic and nicotinic cholinomimetic actions of equal strength. No reference to experiments with the systemic use of nicotinic cholinolytics as anticonvulsants in the presence of a definitely formed kindling phenomenon in the amygdala could be found in the accessible literature.

All the facts described above are evidence that, besides muscarinic cholinergic mechanisms, an important role in the formation of the kindling phenomenon in the amygdala is played also by nicotinic cholinergic mechanisms, for the effects of muscarinic cholinomimetics and of nicotinic cholinolytics are synergic in this test and are considerably potentiated as a result of their combined use. Consequently, in addition to existing modern methods of treatment of epilepsy in man, the use of substances with a marked central nicotinic cholinolytic action also is evidently indicated, and in order to make them more effective, they should be combined with muscarinic cholinomimetics.

Considering the reciprocity of interaction between muscarinic and nicotinic cholinergic mechanisms within a single cholinergic system [3, 4], it can be postulated that blocking muscarinic acetylcholine receptors of the ARAS by methyldiazine depresses its activity effect on the cerebral cortex and thereby weakens the inhibitory tonic effect of the latter on deep brain formations, which leads to facilitation of the spread of excitation from the focus of epileptiform activity and to an increase in severity of the seizures.

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