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THE EFFECT OF OXOTREMORINE AND HARMINE INJECTED IN VIVO ON THE SENSITIVITY TOWARDS ACETYLCHOLINE, NORADRENALINE AND ATROPINE IN VITRO*

INTRODUCTION

Oxotremorine acts as a powerfully cholinergic drug inducing tremor, rigidity, bradykinesia and alteration brainstem monoamines, all symptoms reminiscent of Parkinsonism (3, 12, 13). Tremorine tremorigenesis is a central phenomenon and is the result of excitation of a pacemaker in the tegmental reticular formation at the mesencephalic-pontine junction (14). Harmaline induces in rats and monkeys tremor similar to that of tremorine, bilaterality, sensory inhibition and independence from corticospinal pathways (14, 11). Harmine besides occasionaly induces rigidity and frequently convulsions in a variety of species including primates (5).

The two drugs oxotremorine and harmine induce potent periferal effect at the neuromuscular junction, particularly oxotremorine (4, 6). The evidence of peripheral involement has been demonstrated in genesis of tremor and rigidity produced by oxotremorine (10). Oxotremorine potentiated acetylcholine induced contractures of chronicialy denervated rat hemidiaphragm in vitro.

The results may be explained by mechanism of receptor desentisation (4).

Katz and Miledi have shown that hypersensitivity occured after muscle injury, no matter whether the nerve was present or not (10). These results pointed to the importance of the presence of atrophying or degenerating muscle fibres for the development of hypersensitivity and also suggest that an increase of chemosensitivity can be expected when the muscle fibre is little demaged (15). The hypersensitivity can be greatly reduced within a few hours only by activity of the muscle fibres (12).

Seven days one after another injecting of oxotremorine and harmine to rats and mice induce lasting tremor and changed muscle activity. This activity could be induced by altered chemosensitivity at the muscle

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fibre or at the end-plate. Tremogenic agents could induce hyperactivity or muscle injury and change activity in two directions. The aim of this work is to record sensitivity of different neuromuscular junctions towards acetylcholine, noradrenaline and atropine taken from control animals and animals previously treated seven days with oxotremotine and harmine. The isolated organs used were smooth muscle and striated muscle organs of rats and mice. Two of them are with cholinergic nerve transmission oesophagus and urinary bladder, former with striated muscle and later with smooth muscle. One is ductus deferens (vas deferens) smooth muscle with adrenergic hypogastric nerve.

METHOD

It was used three groups of male rats and male mice. The first two groups of rats and mice were injected i. p. 0,25 mg/kg of oxotremorine. The second two groups of animals were injected 20 mg/kg of harmine i. p. and third two groups were injected with saline. The animals ere stunned by a blow on the head and then decapitated. The abdomen and thorax were opend along the midline and ductus deferens and urinary bladder was exposed. The hypogastric nerve was tied with small peace of peritoneum 1/2 cm from ductus deferens (7). The pelvic nerve was tied around ureter for the pelvic nerves to the bladder run close to the ureter (8). Ductus deferens was prepared only of rats. The heart and lungs were remowed to expose the oesophagus. The longitudinal pair of r a m i o e s o p h a g e i n e r v i v a g i were tied as near as possible to the cranial part of torax (9). The organs were set up in the isolated organe bath containing 40 ml of Tyrode solution. The solution was warmed to 32°±0,5 and bubbled with 95% O_2 and 5% CO_2. The proximal part of ductus deferens and fundus of urinary bladder were tied to frontal lever. The magnification being 4 and the load for the rats organs one gram and for the mice organs 0,5 g. The tied nerves were put through bipolar electrodes as was described[1,16]. Submaximal strength of electrical stimuli 1 msec. duration at a frequency of 40 Hz were applied every min. for 2 sec.

After adaptation period of 10 minutes and record of few control contraction slow infusion of 0,02 ml/min 10^{-4} M of drugs started. The infusion lasted an houre. The drug induced were acetylcholine chloride, noradrenaline chloride and atropine sulphate. Three points were found: pS_m, pB_{75} and pB_{50}, so called pharmacological constants. The first point is pS_m, the place on base line were a molar concentration induced the highest contraction. The second point is pB_{75}, concentration that induced only 75 percent of controle contraction. The third point is pB_{50} molar concentraction of drugs that reduced hight of contraction to the half of the control one. The prefix p means negative logarithm of the molar concentrations. As greater pharmacologic constant as greater chemosensitivity.

RESULTS

A. The effect of electrical stimulation

Electrical stimulation of nerves with trains of pulses caused contraction of oesophagus, urinary bladder and ductus deferens. Constant stimuli produced constant height of contractions in a single preparation. There were not qualitative difference of effect of nerve stimulation between control and test preparation taken from animals previously treated with oxotremorine and harmine.

B. The effect of drugs on responses to electrical stimulation.

1. Infusion of acetylcholine

Acetylcholine caused biphasic response i. e. the increase of the effect of nerve stimulation followed by decrease, depression and block. The organs taken from mice are more sensitive then organs taken from rats. There is no great difference in concentration for increase and decrease of the effect of stimulation, it is less then 5 times molar concentration or less then 0,7 range of value.

Response of the oesophagus taken from the mice pretreated with oxotremorine is less sensitive towards acetylcholine then control organs. The remaining organs with cholinergic nervous supply are more sensitive. The response of ductus deferens to stimulation is increased by acetylcholine in animals treated with oxotremorine (Fig. 1). Harmine pretreatment decreased the sensitivity toward acetylcholine in all three preparations (Tab. 1).

Tab. 1.

THE INFLUENCE OF SLOW INFUSION OF ACETYLCHOLINE (5×10^{-7} M/ml/min.) ON THE RESPONSE OF ISOLATED ORGANS INDUCED BY ELECTRICAL NERVE STIMULATION

Organ	Animal		Seven days injections i. p. Saline	Oxotremorine	Harmine
Oesophagus		pSm	5,68 (6/8)	5,68 (1/6) 5,35 (6/6)	5,61 (2/6) — (0/6)
		pB50	4,97 (3/8)	4,98 (5/6)	— (0/6)
	Mouse	pSm	5,88 (3/6)	4,94 (3/6)	—
		pB75	5,05 (5/6)	4,55 (5/6)	—
		pB50	5,10 (1/6)	4,62 (2/6)	—
Urinary bladder	Rat	pSm	5,34 (5/9)	5,35 (2/6)	— (0/6)
		pB75	5,21 (4/9)	5,32 (4/6)	5,19 (4/6)
		pB50	— (0/9)	4,72 (2/6)	5,07 (1/6)
	Mouse	pSm	5,57 (5/9)	— (0/6)	—
		pB75	4,90 (6/9)	5,75 (6/6)	—
		pB50	4,87 (2/9)	5,42 (2/6)	—
Ductus deferens	Rat	pSm	5,19 (9/9)	5,60 (4/7)	5,11 (6/6)
		pB75	4,88 (3/9)	5,55 (3/6)	— (0/6)
		pB50	4,80 (2/9)	4,81 (3/6)	— (0/6)

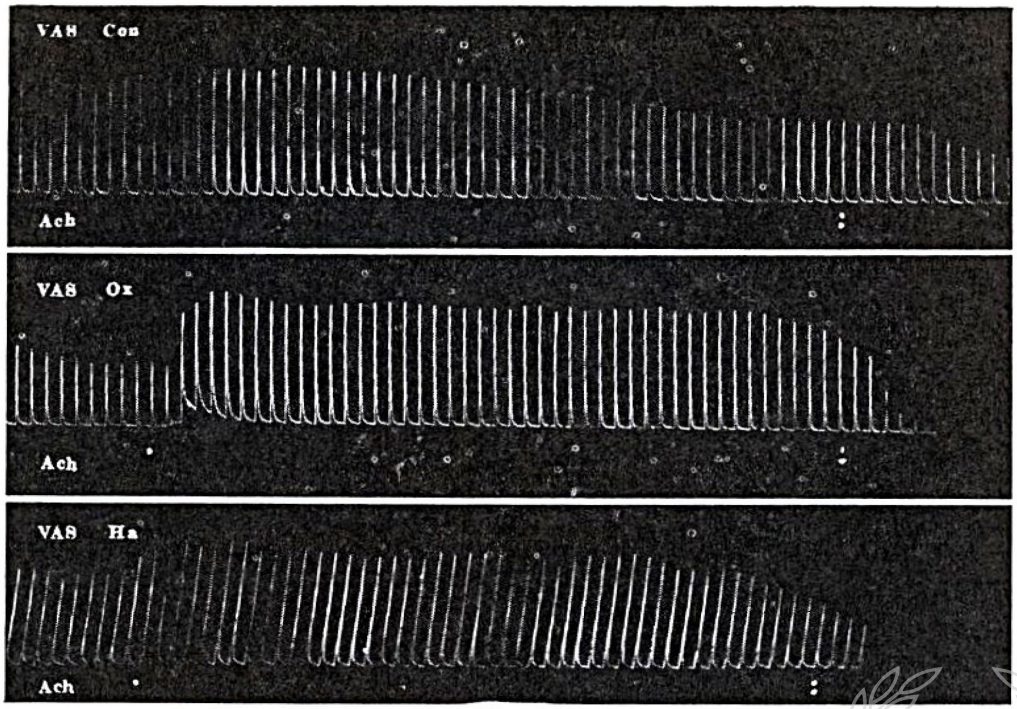

Fig. 1

The contractions of the vas deferens produced the electrical hypogastric nerve stimulation. (5V, 1 mSec, 4 Hz every min. for 2 sec.) At the point acetylcholine infusion started 10—7M/ml/min. The organs were taken from (con) controle (Ox), oxotremorine and (Ha) harmine treated animals.

2. *Infusion of noradrenaline*

Noradrenaline induced biphasic response on ductus deferens. Increase of the effect of nerve stimulation is followed by decrease. The difference in concentration is 0,7 range of value. The same result was recorded on mouse oesophagus. The effect of noradrenaline on other controle organs was decrease of the effect of stimulation.

Tab. 2.

THE INFLUENCE OF SLOW INFUSION OF NORADRENALINE (5×10^{-7} M/ml/min.) ON THE RESPONSE OF ISOLATED ORGANS INDUCED BY ELECTRICAL NERVE STIMULATION

Organ	Animal		Seven days injections i. p. Saline	Oxotremorine	Harmine
Oesophagus	Rat	pSm	— (0/6)	5,03 (3/6)	— (0/6)
		pB75	4,95 (4/6)	4,94 (1/6)	4,47 (2/6)
		pB75	4,52 (1/6)	— (0/6)	— (0/6)
	Mouse	pSm	5,31 (5/6)	5,50 (6/7)	—
		pB75	5,36 (2/6)	4,94 (1/7)	—
		pB50	4,88 (2/6)	4,91 (1/7)	—

Urinary bladder	Rat	pSm	— (0/6)	— (0/6)	5,46 (2/6)
		pB75	5,07 (6/6)	5,31 (3/6)	4,85 (4/6)
		pB50	4,97 (3/6)	5,52 (2/6)	— (0/6)
	Mouse	pSm	— (0/10)	— (0/6)	—
		pB75	5,59 (10/10)	5,88 (4/6)	—
		pB50	5,43 (9/10)	5,65 (4/6)	—
Ductus deferens	Rat	pSm	5,33 (6/8)	— (0/6)	5,70 (4/6)
		pB75	4,89 (5/8)	5,59 (4/6)	5,26 (4/6)
		pB50	4,15 (3/8)	5,45 (4/6)	5,00 (2/6)

Sensitivity of the organs toward noradrenaline taken from the oxotremorine treated animals were increased. There was more pronounced biphasic effect of noradrenaline on oesophagus of animals treated with oxotremorine. Harmine pretreatment decreased sensitivity toward noradrenaline in all preparations with cholinergic nervus supply. Ductus deferens of the rat was more sensitive toward noradrenaline after harmine.

3. Infusion of atropine

Atropine induced increase followed by decrease of the effect of nerve stimulation on striated muscle of oesophagus. Responses of urinary bladder and ductus deferens of controle animals on atropine was decrease of contraction. Organs taken from oxotremorine treated animals did not change sensitivity towards atropine significantly. The same could be recorded for the organs taken from the animals treated with harmine.

Tab. 3.

THE INFLUENCE OF SLOW INFUSION OF ATROPINE (5×10^{-7} M/ml/min.) ON THE RESPONSE OF ISOLATED ORGANS INDUCED BY ELECTRICAL NERVE STIMULATION

Organ	Animal		Seven days injections i. p. Saline	Oxotremorine	Harmine
Oesophagus	Rat	pSm	5,09 (5/6)	5,07 (4/6)	5,41 (4/6)
		pB75	4.72 (4/6)	4,88 (2/6)	— (0/6)
		pB50	4,62 (2/6)	— (0/6)	— (0/6)
	Mouse	pSm	5,71 (4/6)	5,71 (4/6)	—
		pB75	5,27 (5/6)	— (0/6)	—
		pB50	(0/6)	— (0/6)	—
Urinary bladder	Rat	pSm	— (0/10)	— (0/6)	— (0/6)
		pB75	5,70 (10/10)	5,71 (4/6)	5,67 (4/6)
		pB50	5,24 (9/10)	5,32 (4/6)	5,30 (2/6)
	Mouse	pSm	— (0/8)	— (0/6)	—
		pB75	5,89 (8/8)	5,81 (6/6)	—
		pB50	5,66 (8/8)	5,59 (6/6)	—
Ductus deferens	Rat	pSm	5,11 (3/7)	— (0/6)	— (0/6)
		pB75	5,37 (6/7)	— (0/6)	— (0/6)
		pB50	5,27 (5/7)	5,57 (4/6)	5,85 (4/6)

DISCUSSION

The marked qualitative difference of the response to electrical stimulation of the nerve was not recorded on the isolated organs taken from control or test animals. The difference from the, control was found after infusion of acetylcholine and noradrenaline but not after atropine. Chronic treatment with oxotremorine provoked different change of sensitivity of organs taken from mice and rats. It was recorded species differens. Oxotremorine injected seven days one after another increased sensitivity toward acetylcholine and noradrenaline with the exception of the oesophagus of the mice, where was found decrease of the responses.

Chronic treatment with harmine decreased the effect of acetylcholine and noradrenaline on the response of nerve stimulation except on the ductus deferens. The very important exceptions are striated muscle of oesophagus and smooth muscle of vas deferens, former contracted upon the cholinergic vagus nerve stimulation (9) and later responded upon the adrenergic nerve stimulation (7).

Two tremogenic agents injected *in vivo* induced different change toward infusion of drug *in vitro*. It was recorded species differens not only in control animals (16) but in the reactions after oxotremorine and harmine toward acetylcholine and noradrenaline. The results can not be put together and analysed simply. The changes are not in the same direction and slike. The increased sensitivity could be explained by the demage and atrophy of muscle (10) after chronic treatment with oxotremorine. Decreased sensitivity can be explained by hyperactivity, for it is known, the sensitivity can be greatly reduced within a few hours only by activity of the muscle fibre (15), as was induced by tremorgenic substances. It is very probable that the mechanism and the site of activity of tremorgenic substances in perifery is not single ones as well for different agents as for single tremogen.

SUMMARY

The isolated organs with nerves were taken from control and treated rats and mice. Oxotremorine and harmine were injected previously seven days one after another. The nerve vere stimulated every minut with the trains of pulses and contractions of oesophagus, urinary bladder and ductus deferens were recorded. In the bath where isolate organs were set up the slow infusion of acetylcholine, noradrenaline and atropine were started, nad the change of the response were measured and expressed as a negative logarithm of molar concentrations.

The species differences were found. Oxotremorine increased sensitivity towards acetylcholine and noradrenaline with the exception of the oesophagus of mice. Harmine decreased the effect of acetylcholine and noradrenaline on the responses of nerve stimulation except ductus deferens. The two wery important exceptions are striated muscle and

smooth muscle, former contracted upon the stimulation of cholinergic nerve and later upon stimulation of adrenergic nerve. The changes are not in the same directions and slike and the results can not be analysed simply. It is concluded that the mechanism and site of activity are a few in perifery.

M. RADIVOJEVIĆ I S. HUKOVIĆ

PROMJENA OSJETLJIVOSTI PREMA ACETILHOLINU, NORADRENALINU I ATROPINU IN VITRO POD UTICAJEM INJICIRANJA OKSOTREMORINA I HARMINA IN VIVO

KRATAK SADRŽAJ

Promjena osjetljivosti acetilholinskih receptora razne vrste muskulature pod uticajem supstanci i oštećenja bila je poznata odranije. Cilj ovog rada je bio da se ustanovi da li se mijenja senzitivitet prema acetilholinu, noradrenalinu i atropinu pod uticajem sedmodnevnog davanja oksotremorina i harmina. Ispitivanja su vršena na izoliranim organima sa pripadajućim nervima, uzetim od štakora i miša koji su prethodno sedam dana uzastopice injicirani oksotremorinom (0,25 mg/kg) i harminom (20 mg/kg). Nervi su stimulirani svake minute i registrovane su kontrakcije glatkih, odnosno poprečnoprugastih mišića. Upoređeni rezultati su dobiveni na kontrolnim i tretiranim preparatima i izraženi u vrijednostima $p^{S}m$, $p^{B}75$ i $p^{B}50$. Nije nađena velika razlika, mada postoji signifikantna razlika. Rezultati su kompleksni i ne mogu se staviti skupa i jednostavno se analizirati, jer postoje značajni izuzeci. Razlika je nađena u osjetljivosti prema acetilholinu i noradrenalinu, a ne prema atropinu. Oksotremorin povećava osjetljivost prema acetilholinu i noradrenalinu, — izuzetak je ezofagus štakora. Nađena je također razlika između vrsta, tj. drugačije se mijenja osjetljivost kod štakora neko kod miša. Harmin smanjuje osjetljivost prema acetilholinu i noradrenalinu, osim na duktus deferensu štakora. Smanjenje osjetljivosti se tumači kao posljedica hiperaktiviteta, a povećanje senzibiliteta — kao rezultat oštećenja i atrofije. Vjerovatno je da nisu jedinstveni mehanizam i mjesto djelovanja tremogenih supstanci na periferiji.

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