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RECEPTOR MECHANISMS IN VASOCONSTRICTOR RESPONSES TO CHOLINE ESTERS IN *BUFO MELANOSTICTUS*

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Abstract: Choline esters have been found to cause vasoconstriction of perfused systemic blood vessels of common Indian toad, *Bufo melanostictus*. The vasoconstriction appears to be mediated through atropine sensitive receptors and nicotinic mechanisms appear unlikely to be involved.

Key words: receptors, cholinergic, blood vessels, evolution, physiology, amphibia

INTRODUCTION

The vasculature in most species responds to cholinergic drugs with vasodilatation despite an apparent lack of cholinergic innervation of most blood vessels (1). In the Indian frog (*Rana tigrina*), vasoconstriction is seen with low doses of acetylcholine, and many other choline-esters (2). This was reported to be a muscarinically mediated phenomenon. Another amphibian, *Bufo melanostictus*, the common Indian toad is used as an alternative to the frog in many physiological and pharmacological experiments. It was, therefore, decided to evaluate the behaviour of the blood vessels of the toad towards the cholinergic drugs and to elucidate receptor mechanisms involved.

METHODS

The systemic blood vessels of pithed toads (*Bufo melanostictus*), were perfused with Frog Ringer solution at ambient temperature and pressure of 30-50 cm of water by gravity feed. The perfusion pressure and the effects on the same by injected drugs were recorded, as described elsewhere (2). The agonist responses were expressed as percent vasoconstriction or vasodilatation, and effects of interacting drugs as their percent block or potentiation of original response.

The drugs used were chloride salts of acetylcholine (Ach) carbachol (Cch), methacholine (Mch), bethanechol (Bch, all Koch-light, U.K.), butyrylcholine (Buch, Sigma, USA), barium (Ba, BDH, India), and propionylcholine iodide (Prch, Sigma, USA), adrenaline tartrate (AD, Mukti Pharma, India), tyramine hydrochloride (TY, Sigma, USA), dimethylpheny lpiperazinium iodide (DMPP, Fluka, Switzerland) AHR-602 (N-benzyl-3 pyrrolidylacetate methobromide A.H. Robins, U.K.), atropine sulphate (AT, E. Merck Germany), co-dergocrine mesylate or 'Hydergine (Hyd, Sandoz, India), and mecamylamine hydrochloride (Meca, Sigma, USA).

The results were analysed by paired 't' test.

RESULTS

Effects of drugs per-se : The pooled data of the responses of respective drugs are summarised in Table-I. All the choline-esters produced mild to moderate vasoconstriction of quick onset and short but varying duration (1 to 25 min depending on dose). The effects of Ach and Cch were relatively more marked than those of other drugs. Bch, Prch, and Buch were relatively less potent. The vasoconstriction, which was partial, reached plateau at the dose of 50 µg/100 g body wt in case of Ach, Cch and Mch, and at the dose of 500 µg/100 g body wt in case of Bch and Prch. The effect of Buch declined sharply as the dose was

increased. The administration of drugs was followed by skeletal muscle faciculations which were visually evident with higher doses of Ach, Cch, Prch and Buch.

AD produced vasoconstriction of quick onset and short duration but the effect did not exhibit dose-dependence. DMPP produced vasoconstriction that was similar to AD though less intense. TY and AHR-602 did not show any effect. Ba produced marked vasoconstriction of quick onset and shorter duration.

Drug Interactions:

(a) Effect of ganglion blocker :Meca pretreatment in the doses of 50 µg/100 g body wt caused moderate to marked block of vasoconstrictor effects of Ach, Cch, Mch and Bch as well as of DMPP. The blockade of Cch and Bch was more marked than those of Ach and Mch. The effects of AD and Ba were, however, slightly or not at all affected (Table-II).

TABLE I : Effects of some vasoactive drugs *per se* on the perfused systemic blood vessels of *B. melanostictus*. Data represents % (Mean ± SE) constrictor effect. Figures in parentheses indicate *n*.

Drugs	*Dose/100 g body wt*						
A: Dose in µg	1	2	5	10	25	50	100
Ach	8.41****	11.47****	10.46****	15.24****	17.42****	20.26***	–
	±0.79	±1.49	±1.28	±1.39	±1.45	±1.19	
	(90)	(91)	(91)	(110)	(147)	(209)	
Cch	4.06***	7.24****	7.61****	6.98****	16.12****	20.49****	–
	±0.98	±1.45	±1.36	±1.02	±1.76	±1.76	
	(52)	(50)	(56)	(57)	(98)	(121)	
Mch	4.09^{NS}	6.86*	6.95**	10.26***	11.35****	–	–
	±2.47	±2.99	±2.54	±3.11	±1.45		
	(21)	(21)	(22)	(23)	(60)		
DMPP	–	–	–	–	16.0***	11.66****	11.91****
					±2.60	±1.80	±1.78
					(10)	(12)	(12)
AD	28.86****	22.16****	32.03****	–	–	–	–
	±2.11	±0.87	±0.72				
	(64)	(212)	(429)				
B : Dose in mg	0.05	0.1	0.2	0.5	1.0	2.0	10.0
Bch	–	7.58****	7.95****	11.89****	12.21****	6.37****	–
		±1.06	±1.66	±1.91	±1.75	0.95	
		(19)	(19)	(19)	(86)	(86)	
Prch	9.54****	12.0***	7.29**	12.25^{NS}	5.33^{NS}	6.67***	–
	±1.28	±3.14	±2.38	±5.05	±1.33	±0.66	–
	(24)	(11)	(7)	(4)	(3)	(3)	
Buch	–	–	–	18.05****	15.14***	4.33***	
				±2.00	±3.28	±0.33	–
				(19)	(7)	(3)	
TY	–	–	–	–	–	–	±1.0^{NS}
							±2.75
							(12)
Ba	–	–	–	–	–	–	43.37****
							±1.43
							(347)

*P<0.05; **P<0.02; ***P<0.01; ****P<0.001 (by paired 't' test) ; NS = Not significant.

(b) Effect of α-adrenergic blocker : Hyd pretreatment in the doses of 60 μg/100 g body wt caused mild to moderate blockade of vasoconstrictor effects of Ach, Cch, Mch and Bch, as well as of AD and marked blockage of the effects of DMPP. The effect of Ba were also blocked (Table II).

TABLE II : Interaction of some antagonists with vasoactive agents on perfused systemic blood vessels of *B. melanostictus*. Data represents % (Mean±SE) block of initial vasoconstriction. Figures in parentheses represent *n*.

Drugs and dose (per 100 g)		*Mecamylamine (50 μg/100 g. body wt)*	*Hydergine (60 μg/100 g body wt)*
Ach	25 μg	48.75 ± 9.12**** (33)	–
	50 μg	53.24 ± 7.34**** (33)	23.39 ± 8.16*** (28)
	100 μg	–	22.68 ± 8.55** (28)
Cch	25 μg	78.56 ± 4.74**** (23)	–
	50 μg	78.00 ± 5.88**** (23)	45.14 ± 5.27**** (22)
	100 μg	–	20.81 ± 8.49** (21)
Mch	25 μg	50.10 ± 8.82**** (19)	–
	50 μg	43.80 ± 10.03**** (20)	48.18 ± 9.85**** (22)
	100 μg	–	53.27 ± 8.98**** (22)
Bch	1 mg	85.00 ± 5.26**** (22)	49.36 ± 16.85*** (19)
	2 mg	87.00 ± 5.79**** (22)	21.65 ± 21.25^{NS} (20)
DMPP	100 μg	44.70 ± 2.71**** (112)	85.93 ± 3.95 **** (15)
AD	2 μg	–	65.06 ± 2.43**** (92)
	5 μg	12.74 ± 5.47* (89)	58.84 ± 2.58**** (92)
Ba	10 mg	5.35 ± 5.41^{NS} (88)	23.63 ± 4.89**** (92)

*P<0.05; **P<0.02 ; ***P<0.01; ****P<0.001 (by paired 't' test) NS = Not significant.

(c) Effect of antimuscarinic agent : AT pretreatment in the doses of 80 µg/100 g body wt. caused almost complete block of the vasoconstrictor effects of Ach, Cch, Mch and Bch, without affecting that of AD. The Ba effects were slightly potentiated, while DMPP effects were partially inhibited. On increasing the dose of choline-esters the degree of blockade was progressively reduced in each case.

DISCUSSION

Choline-esters with known muscarinic activity, namely Ach, Cch, Mch and Bch caused vasoconstriction of the systemic blood vessels of *Bufo melanostictus,* in contrast to the vasodilatation reported in most other species (1). Similar vasoconstriction was observed in *Rana tigrina,* another amphibian species (2).

The vasoconstrictor effects of the choline-esters in *B. melanostictus* were partially blocked, but more prominently than in *R. tigrina,* by Meca as well as Hyd, in doses which caused moderate blockade of respective specific agonists, DMPP and AD. Further, Hyd showed significant block of Ba effects, in contrast to results in *R. tigrina,* indicting some nonspecificity of action even in submaximal blocking doses. AT, blocked the vasoconstrictor actions of all the four choline-esters, which is in general agreement with earlier reports on *Rana tigrina* (2).

The absence of the response to AHR-602 might indicate relative absence of ganglionic muscarinic excitator mechanisms in *Bufo melanostictus*, as are known in the case of *Rana tigrina* (2, 3). The observations in general reflect more of quantitative rather than qualitative difference, between vasoconstrictor cholinergic mechanisms of *R. tigrina* and *B. melanostictus.* The vasoconstrictor cholinergic mechanisms are also reported in fishes (4). Such a response does not appear in mammals, indicating the transitional characteristics between aquatic and terrestrial phylogenetic spectra.

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