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SOME ASPECTS OF VASCULAR PHARMACOLOGY OF FROG *(RANA TIGRINA)*

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Abstract: Vascular autonomic receptors in amphibians exhibit difference from more evolved mammalian species. Vascular perfusion studies in frog indicate constrictions by prominent muscarinic but rudimentary nicotinic constrictive regulation by cholinergic systems. Difference from classical effect-patterns of pharmacological interventions, observed in the study, make room to visualise complexity of additional regulatory mechanisms.

Key words: receptors cholinergic blood vessels amphibia evolution physiology

INTRODUCTION

The choline-esters dilate mammalian vascular beds acting upon muscarinic receptors directly with no apparent involvement of cholinergic innervation and such effect is blocked by low doses of atropine (1), while higher doses exert vasoconstriction mostly by nicotinic mediation (2, 3, 4). In frog, however, muscarinic vasoconstrictor responses to low doses of acetylcholine and other choline-esters (5, 6, 7) were detected. The autonomic mechanisms in amphibians thus appear to be dissimilar to those in mammalian species. The present communication incorporates observations of further experiments attempting to delineate dose response characteristics to qualitatively and quantitatively differing agonist and antagonists of autonomic function, with reference to the vasculature in frog.

METHODS

Perfusion experiments on systemic blood vessels in pithed frogs (*Rana tigrina*, either sex, weighing 80-150 g) were conducted. One of the aortae was perfused with frog Ringer solution (NaCl 6.5, Kcl 0.14, $CaCl_2$ 0.12, $NaHCO_3$ 0.2, NaH_2PO_4 0.01 g/l) at room temperature and adjusting perfusion pressure of 30-50 cm column by gravity feeding. The other aorta was ligated and the heart was removed to allow free outflow of the perfusate from vena cava. The perfusion pressure was monitored and changes expressed as percent vasoconstriction or vasodilatation and degree of their blockade or potentiation. The drugs were injected in perfusion tube in constant volume of 0.5 ml and effect of this volume was recorded for subsequent correction of data. Details of the method are reported elsewhere (8).

The drugs used were, acetylcholine chloride (Ach), carbacholine chloride (Cch), methacholine chloride (Mch), betamethyl choline chloride (Bch, all Koch-Light, U.K.), butyrylcholine chloride (Buch, Sigma, USA), barium chloride (Ba, BDH, India), and propionylcholine iodide (Prch, Sigma, USA); adrenaline tartrate ± (AD, Mukti Pharma, India), tyramine hydrochloride (TY, Sigma, USA), dimethylphenyl piperazinium iodide (DMPP, Fluka, Switzerland), AHR-602 (N-benzyl-3 pyrrolidylacetate methabromide, A.H. Robins, U.K.), atropine sulfate (AT, E. Merck, Germany), codergocrine mesylate (Hyd, Hydergine, Sandoz, India), and mecamylamine hydrochloride (Meca, Sigma, USA).

The drug solutions were made either fresh or from refrigerator stocks less than a week old.

The results were analysed by the paired 't' test.

RESULTS

Effects of drugs *per-se:*

Table I : All the choline-esters produced vasoconstriction of rapid onset (duration 4-25 min depending on dose). The effect of Ach, Cch and Mch were relatively more marked than those of other drugs. Bch, Prch and Buch were relatively less potent. The vasoconstriction exhibiting plateau effect at the dose of 50 µg/100 g in case of Ach, Cch and Mch, and at 500 µg/100 g in dose of Bch and Prch. The effect of Buch declined sharply as the dose was increased. The injection of drugs was followed by skeletal muscle spasm which were visually evident with higher doses of Ach, Cch, Mch, Prch and Buch.

Vasoconstrictive responses to different choline-esters lasted 4-25 min directly depending on the dose

TABLE I : Effects of some vasoactive agents *per se* on the perfused systemic blood vessels of *R. tigrina*. Data represents % (Mean±SE) vasoconstrictor effect. Figures in parentheses indicate n.

Drugs	Dose/100 g body wt								
A : Dose in µg	**1**	**2**	**5**	**10**	**25**	**50**	**100**	**200**	**500**
Ach	21-25**** ±2.26 (16)	25.25**** ±2.52 (16)	24.42**** ±2.36 (26)	31.59**** ±3.51 (27)	32.49**** ±2.06 (55)	39.68**** ±1.95 (90)	43.37**** ±2.17 (54)	44.0**** ±3.10 (32)	53.87**** ±4.54 (16)
Cch	10.78**** ± 1.36 (18)	14.50**** ±1.62 (18)	25.07**** ±2.06 (29)	29.72**** ±3.15 (29)	33.39**** ±2.29 (48)	40.92**** ±2.50 (53)	35.53**** ±2.83 (34)	36.55**** ±3.85 (18)	32.22**** ±3.31 (18)
Mch	11.07*** ±2.93 (15)	15.0*** ±4.12 (14)	22.0**** ±3.50 (24)	28.92**** ±4.58 (20)	31.73**** ±3.00 (37)	37.81**** ±2.81 (53)	36.10**** ±3.63 (30)	29.21**** ±5.46 (14)	35.36**** ±6.89 (14)
DMPP	–	–	–	–	9.33*** ±2.32 (6)	5.33** ±1.54 (6)	6.50*** ±1.54 (6)	6.00* ±2.01 (6)	6.16*** ±1.05 (6)
AD	25.52**** ±2.91 (31)	32.51**** ±1.79 (101)	41.88**** ±1.28 (224)	–	–	–	–	–	–
B : Dose in mg	**0.05**	**0.1**	**0.2**	**0.5**	**1.0**	**2.0**	**10.0**		
Bch	–	4.00^{NS} ±1.95 (14)	6.86*** ±2.20 (14)	17.64*** ±4.34 (14)	22.57**** ±2.62 (49)	23.02**** ±2.83 (49)	–		
Prch	16.0*** ±2.92 (20)	22.71**** ±3.46 (7)	29.0*** ±2.64 (3)	28.67*** ±1.66 (3)	23.0* ±4.58 (3)	19.0* ±4.16 (3)	–		
Buch	–	–	–	40.95*** ±4.78 (19)	19.17*** ±4.06 (6)	3.33* ±0.66 (3)	–		
TY	–	–	–	–	–	–	6.15^{NS} ±4.25 (13)		
Ba	–	–	–	–	–	–	40.35**** ±1.78 (156)		

*P<0.05; **P<0.02; ***P<0.1; ****P<0.001 (by paired 't' test); NS - Not significant

of agonists used. In repeated studies with same dose, soon after reversal of vasoconstrictive responses of choline-esters, Cch and Buch exhibited decremental effects within 10 repetitions. Other agonists did not show such decrements within 10 repetitions. The above decrement of responses to Cch and Buch are indicative of tachyphylaxis. All experiments with agonists alone or along with antagonists were performed requiring less than 10 repeated doses. Individual agonists were studied in each animal set up separately when repeat dosing was needed.

AD produced vasoconstriction of immediate onset and duration 6-25 min depending upon the dose used. AD was, dose for dose, more potent than Choline-esters. DMPP produced vasoconstriction that was less intense and not dose dependent. TY and AHR-602 did not show any effect. Ba showed marked vasoconstriction of rapid onset with duration of 20-45 min.

Drug Interactions

Interaction experiments of antagonist were done with many agonists, but agonist dose administrations were restricted to two pre-treatment and two post-treatment doses only in relation to its antagonist. The effect of antagonist is presented as % blockade (Table II) of the % vasoconstrictive effects of agonists (Table I).

a) Effect of ganglion blocker: Meca in a single dose of 50 µg/100 g was used to assess purely qualitative interaction with vasoconstrictor influence of various agonists. *Per se* injection of Meca caused a minor vasodilatation, which spontaneously waned in 3-4 min. The blocking interaction was assessed only after such reversal and retention of base line. It was found that single dose administration of Meca was adequate to study the qualitative blockade of three agonists in repeat doses involving reversed sequence in a single experiment.

Meca (50 µg/100 g) caused a mild to moderate block of vasoconstrictor effects of Ach, Cch, Mch and Bch as well as of DMPP. The blockade of Bch was more marked than those of Ach, Cch and Mch. The effects of AD were significantly potentiated while Ba responses were not affected at all (Table II).

b) Effect of α-adrenergic blockade : Hyd in the doses of 60 µg/100 g caused a mild to moderate blockade of vasoconstrictor effects of Ach, Cch, Mch and Bch as well as of DMPP and markedly blocked the effects of AD. Ba responses were not affected (Table II).

c) Effect of anti-muscarinic agents: AT in the doses of 80 µg/100 g caused almost complete block

TABLE II : Interaction of some antagonists with vasocative agents on perfused systemic blood vessels of *R. tigrina*. Data represents % (Mean±SE) block or potentiation (+) of initial vasoconstriction. Figures in parentheses represent *n*.

Drugs and dose (per 100 g)	*Mecamylamine (50 µg/100g body wt)*	*Hydergine (60 µg/100 g body wt)*
Ach 25 µg	30.05±11.97** (19)	–
50 µg	41.95±7.88**** (19)	16.18±7.26* (22)
100 µg	–	17.95±7.41** (22)
Cch 25 µg	21.79±5.59*** (19)	–
50 µg	30.95±7.49**** (19)	26.25±6.29**** (16)
100 µg	–	28.67±7.39*** (15)
Mch 25 µg	34.71±10.89*** (17)	–
50 µg	31.41±7.99*** (17)	43.12±3.90**** (16)
100 µg	–	40.25±3.76**** (16)
Bch 1 mg	67.53±9.12**** (19)	67.56±9.18**** (16)
2 mg	55.10±11.79**** (19)	31.06±14.64* (16)
DMPP 100 µg	56.92±3.46**** (74)	67.58±6.25**** (12)
AD 2 µg	–	81.87±2.52**** (70)
5 µg	+19.85±7.31**** (74)	70.47±3.33**** (70)
Ba 10 mg	+1.61±4.65^{NS} (74)	+4.51±5.41^{NS} (67)

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

of the vasoconstrictor effects of Ach, Cch, Mch and Bch without affecting that of AD. The DMPP effects were significantly inhibited while that of Ba were not affected.

Increasing doses of choline-esters tended to progressively reduce the blockade induced by Atropine. This is apparent in comparative effect of small vs. large doses in Table III.

Ach in *R. tigrina* is reported (5,6,7) and is confirmed by these observations.

The vasoconstrictor effects of cholinergic drugs were partially antagonised by mecamylamine and similar doses moderately blocked the vascular responses to specific nicotinic agonists i.e. DMPP and adrenaline. However, the blocks reveal no quantitative discrimination between nicotinic cholinergic influence of drugs

TABLE III : Interaction of some vasoactive agents with Atropine (80 μg/100 g body wt) on perfused systemic blood vessels of *R. tigrina*. Data represents % (Mean±SE) block or potentiation (+) of initial vasoconstriction. Figures in parentheses represent *n*.

Drugs	*Dose/100 g body wt*								
A : μg	1	2	5	10	25	50	100	200	500
Ach	99.37 ±0.44 (16)	99.37 ±0.44 (16)	95.81 ±3.55 (16)	94.50 ±2.99 (16)	93.44 ±3.34 (16)	90.06 ±3.69 (16)	79.37 ±5.98 (16)	72.37 ±5.05 (16)	59.56 ±6.59 (16)
Cch	98.89 ±0.68 (18)	98.89 ±0.56 (18)	98.89 ±0.58 (18)	97.78 ±0.78 (18)	90.17 ±2.75 (18)	84.94 ±3.03 (18)	75.78 ±3.72 (18)	67.28 ±4.14 (18)	63.89 ±4.03 (18)
Mch	100.00 ±0.00 (14)	100.00 ±0.00 (14)	100.00 ±0.00 (14)	99.92 ±0.07 (14)	98.71 ±0.68 (14)	94.86 ±1.44 (14)	89.85 ±1.84 (13)	86.36 ±1.73 (14)	79.71 ±3.28 (14)
DMPP	–	–	–	–	–	–	56.75 ±9.93 (12)	–	–
AD	17.09^{NS} ±8.82 (31)	11.19^{NS} ±7.78 (31)	9.06^{NS} ±7.18 (31)	–	–	–	–	–	–
B : mg	0.1	0.2	0.5	1.0	2.0	10.0			
Bch	100.00 ±0.00 (14)	100.00 ±0.00 (14)	95.21 ±1.86 (14)	67.00 ±8.36 (14)	46.71 ±9.67 (14)	–			
Ba	–	–	–	–	–	+2.67NS ±12.84 (12)			

P value for all blockades <0.001 (by paired 't' test), NS = not significant

DISCUSSION

Acetylcholine and other muscarinic cholinergic drugs viz. acetylcholine, carbachol, methacholine and bethanechol induced vasoconstriction in the systemic vasculature of *Rana tigrina* in contrast to vasodilatatory influence observed in vascular bed of most mammalian species (1). The vasoconstrictor effect of like acetylcholine and carbachol and the pure muscarinic agonists methacholine and bethanechol. Vascular responses to barium lacked any influence of the nicotinic blockers which suggests that nicotinic receptor involvement in vasoconstrictor action of cholinergic drugs is yet equivocal. Further, skeletal muscle spasms do not appear to contribute significantly to the vasoconstrictor effects of predominently muscarinic

choline-esters, it is the acetylcholine and carbachol and infrequently methacholine and bethanechol which were active.

Atropine uniformly blocked the constrictor actions selectively of all the four choline-esters. However, higher doses of all the choline-esters always tended to reverse the atropine blockade, indicating more specific involvement of muscarinic component, suggesting further that nicotinic component were unlikely to be involved. The present study partly confirms earlier reports in *R. tigrina* (5) which observed partial blockade of acetylcholine by atropine and is in sole agreement with the results (7).

The absence of any vascular effect of AHR-602 is in agreement with reported paucity of excitatory muscarinic ganglionic mechanisms in *R. tigrina* (7,9). Vasoconstrictor cholinergic mchanisms are known to exist also in fishes (10) but not in mammals. These observations therefore, might reflect evolutionary transition of tissue pharmacology.

DMPP produced very small vasoconstrictive responses, despite quite high doses, which may reflect a poor suitability of this ganglion stimulant for such vascular studies. The same appears to be true for the interaction of different blockers against DMPP. However, from the above studies it appears that some constrictor cholinergic function in addition to adrenergic constrictor mechanisms exists together in the studied amphibian species.

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REFERENCES

1. Taylor P. Goodman & Gilman's The Pharmacological basis of therapeutics, Eds. Gilman AG, Rall TW, Nies AS, Taylor P. Vol. I, 8th Edn, Pargamon, New York, 1990, p 122-130.
2. Kottegoda SR. The action of nicotin and acetylcholine on the vessels of the rabbit ear. *Br J Pharmacol* 1953; 8:156-161.
3. Beleslin DB. Action of acetylcholine on the blood pressure of the rat. *Arch Int Pharmacodyn Ther* 1963; 141:532-542.
4. Burn JH, Rand MJ. Noradrenaline in artery walls and its dispersal by reserpine. *Br Med J* 1958; i: 903-908.
5. Rahman SA. Action of acetylcholine, noradrenaline and some of the autonomic blocking agents on the perfused blood vessels of the frog. *Indian J Physiol Pharmacol* 1963; 7:80-91.
6. Gambhir SS, Das PK. Effect of acetylcholine and carbachol on the blood vessels of the frog. *QJ Exp Physiol* 1970; 55:313-319.
7. Gambhir SS, Das PK. Cholinergic receptor mechanisms in amphibian vasoconstrictor responses. *Pharmacology* 1978; 16:115-120.
8. Gambhir SS, Das PK. Modified technique for recording perfusion rate. *J Pharm Pharmac* 1970a; 22:788-789.
9. Libet B. Slow synaptic responses and excitatory change in sympathetic ganglia. *J Physiol Lond* 1964; 174:1-25.
10. Burnstock G. Evolution of the autonomic innervation of visceral and cardiovascular systems in vertebrates. *Pharmacol Rev* 1969; 21:247-324.