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COMMUNICATIONS

Adrenergic presynaptic receptors: an overextended hypothesis?

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Based on a historically fruitful model of autonomic receptors located on or in responding smooth and cardiac muscle cells, the hypothesis of inhibitory adrenergic presynaptic sites modulating neurotransmitter release has achieved a rapid and distinctive status (Rand et al 1975; Starke & Endo 1976; Langer 1977; Starke 1977). Derived in essence from manifold observations in numerous species and tissues that phenoxybenzamine and allied α-receptor antagonists enhance, and noradrenaline and its analogues reduce the efflux of sympathetic nerve transmitter during stimulation it appears to provide a unitary explanation of neuronal events involving exogenous agonists and antagonists. This attractive hypothesis, introducing parsimony and feedback precision into the exocytotic release process remains, however, untested in its particulars. The negative feedback function assigned to the neuronal α-adrenoceptors makes demands on its performance which can be, but have not yet been, tested experimentally.

Guinea-pig left atria were bisected and incubated for 60 min in 4·0 ml of oxygenated (5% CO_2 in O_2) Krebs-Henseleit (Krebs) solution (Kalsner 1979a) containing (−)-[7,8-^{3}H]noradrenaline (10 μCi ml^{-1}, 7·6–10·0 × 10^{-7} M) then washed with fresh Krebs solution and mounted under 2 g tension between platinum wire electrodes. Tissues were superfused continuously with warmed (37°C) and oxygenated Krebs solution at a flow rate of 5 ml min^{-1}. Cocaine hydrochloride (3 μg ml^{-1}; $8·8 \times 10^{-6}$ M) and normetanephrine hydrochloride (2·2 μg ml^{-1}; 1×10^{-5} M) were routinely present in the Krebs solution to block neuronal and extraneuronal uptake processes. Phenoxybenzamine hydrochloride and (−)-noradrenaline bitartrate, when used, were dissolved directly in the Krebs solution.

After a 90 min equilibration period each tissue was stimulated transmurally with a train of 100 pulses of 1 ms duration and supramaximal voltage once at each of the five test frequencies (0·5–10 Hz) in random sequence, with a 10 min interval between tests. One of each pair of atrial halves was then exposed to either the agonist (20 min) or antagonist (30 min) followed by a repetition of the stimulation cycle in both the control and treated preparations.

The efflux of [^{3}H]noradrenaline from the preparations was determined by counting 1·0 ml aliquots of the 15·0 ml superfusate collected in vials by a fraction collector which rotated every 3 min. The aliquots were transferred to vials containing 10 ml of Aqueous Counting Scintillant (Amersham) and counted to a 1% error in a Beckman LS-230 counter with automatic external standardization to determine efficiency. Basal efflux is expressed as disintegrations min^{-1} (d min^{-1}) and referred to as the total radioactivity detected in the 3 min sample collected immediately before stimulation. Stimulation induced efflux was calculated as the difference between basal efflux and the total d min^{-1} in the 3 min samples collected during and immediately after stimulation. Transmural stimulation was always begun at the onset of a 3 min collection period. Mean data are presented with their standard errors and Student's t-test was used for all comparisons with a P value of less than 0·05 considered significant.

If endogenously released noradrenaline activates a negative feedback loop, mediated by presynaptic α-receptors, to inhibit subsequent transmitter output, it can be predicted that, as the concentration of neurally released transmitter in the synaptic gap increases with increasing frequency of stimulation, the proportional contribution of a fixed quantity of exogenous noradrenaline to the total noradrenaline incident on the receptors must decline. This was tested in the experiments recorded here, by compressing the release process with 100 pulses, from a total time of 200 s to a minimum of only 10 s. The results shown in Table 1 demonstrate that exogenous noradrenaline reduces the stimulation-induced efflux of tritium, having its most effect at the lower and its least effect at the higher test frequencies, seemingly in accord with the expectations of presynaptic receptor theory. This superficial correspondence is not sustained with closer examination. Firstly, the total efflux of tritium with 100 pulses in initial tests on all atria in the absence of noradrenaline should decline with rising frequency, the consequence of an increasingly activated autoinhibitory system in the presence of an increasing perineuronal level of transmitter. This was not seen. The total efflux of tritium

* Correspondence.

Table 1. The ratios of transmitter overflow in the first and second periods of field stimulation of guinea-pig atria in the absence and presence of noradrenaline (n = 6).

Group	Hz	Transmitter overflow 1st stim. period ($\times 10^3$ d min^{-1})	Overflow ratio 2nd period 1st period	% of Control
Control	0·5	9·05±1·82	0·87±0·10	
NA[a]	0·5	11·00±2·06	0·19±0·07**	19·15±5·93(a)
Control	1	20·70±3·00	0·94±0·11	
NA[a]	1	22·80±2·87	0·15±0·05**	13·83±3·23(b)
Control	2	22·80±2·62	0·94±0·08	
NA[a]	2	30·25±2·75	0·20±0·05**	20·47±3·46(c)
Control	5	26·02±4·05	0·87±0·14	
NA[a]	5	33·70±2·97	0·30±0·07*	34·83±3·67(d)
Control	10	22·20±2·75	1·06±0·10	
NA[a]	10	29·25±2·13	0·48±0·11*	44·28±7·66(e)

* $P<0·01$; ** $P<0·001$ compared with ratio for control group. [a]Strips were exposed to noradrenaline (3×10^{-6} M) after the initial period of stimulations with 100 pulses at each test frequency followed 20 min later, without washout, by a repetition of the stimulations. (a) vs (b) NS; (a) vs (c) NS; (a) vs (d) $P<0·05$; (a) vs (e) $P<0·05$; (b) vs (c) NS; (b) vs (d) $P<0·01$; (b) vs (e) $P<0·01$; (c) vs (d) $P<0·02$; (c) vs (e) $P<0·02$; (d) vs (e) NS.

Table 2. The ratios of transmitter overflow in the first and second periods of field stimulation of guinea-pig atria in the absence and presence of phenoxybenzamine (n = 8).

Group	Hz	Transmitter overflow 1st stim. period ($\times 10^3$ d min^{-1})	Overflow ratio 2nd period 1st period	% of Control
Control	0·5	13·20±2·11	0·84±0·10	
POB[a]	0·5	16·61±1·69	1·96±0·30**	242·73±36·37(a)
Control	1	30·64±4·54	0·88±0·07	
POB[a]	1	36·71±3·25	2·12±0·19***	242·04±14·21(b)
Control	2	36·74±5·24	0·88±0·05	
POB[a]	2	47·14±4·54	2·05±0·18***	231·26±11·57(c)
Control	5	40·76±4·78	0·88±0·04	
POB[a]	5	52·54±3·86	1·55±0·11***	175·76±4·95(d)
Control	10	52·65±6·83	0·89±0·07	
POB[a]	10	63·19±6·65	1·18±0·12*	132·66±12·57(e)

* $P<0·5$; ** $P<0·01$; *** $P<0·001$ compared with ratio for control group. [a]Phenoxybenzamine (1×10^{-5} M) was administered for 30 min after the initial period of stimulations with 100 pulses at each test frequency followed, 20 min after its washout, by a repetition of the stimulations. (a) vs (b) NS; (a) vs (c) NS; (a) vs (d) $P<0·1>0·05$; (a) vs (e) $P<0·02$; (b) vs (c) NS; (b) vs (d) $P<0·001$; (b) vs (e) $P<0·001$ (c) vs (d) $P<0·001$; (c) vs (e) $P<0·001$; (d) vs (e) $P<0·01$.

differed only fractionally, and in fact, tended to increase in untreated atria (Tables 1 & 2) when the frequency of stimulation ranged between 1 and 10 Hz and was much less at 0·5 Hz than at the other test frequencies.

Secondly, the contraction of the stimulation period from 200 to 100s (from 0·5–1·0 Hz) coupled with a doubling in the absolute output of ^{3}H-transmitter should have produced a pronounced elevation in the ambient concentration of active transmitter, making the contribution of a fixed amount of foreign noradrenaline a less significant component of the total incident noradrenaline. However, the inhibition of efflux induced by the added agonist did not differ significantly between 0·5 and 1·0 Hz (Table 1). Further, the percent inhibition by noradrenaline at 0·5, 1·0 and 2·0 Hz did not differ significantly from each other nor did that between 5 and 10 Hz. The general attrition in the potency of noradrenaline when efflux ratios at 0·5, 1 and 2 Hz are compared with 5 and 10 Hz does not seem to correspond with the magnitude of the probable changes in the perineural level of transmitter (Table 1) and suggests that another explanation should be sought.

The effect of phenoxybenzamine on stimulation-induced efflux was fundamentally discrepant with that predicted for an autoinhibitory system rendered non-functional by a covalently bound antagonist. Blockade of sites, which would otherwise be increasingly activated by endogenously liberated noradrenaline as the frequency of stimulation climbed, should have led to a progressive magnification of the difference between untreated and treated preparations in stimulation-induced efflux. This clearly was not observed (Table 2). Instead the effect of the antagonist declined generally over the frequency range 0·5–10·0 Hz and showed a stable level of efflux enhancement at the three lower frequencies: an insensitivity to the changing ambient concentration of transmitter discordant with its postulated mechanism of action. Further, the present findings demonstrate no reciprocal pattern between the effects of noradrenaline and phenoxybenzamine, requisite for their interaction with presynaptic sites as agonist and antagonist. What is apparent instead is a commonly observed profile of effect: drugs which interact with neuronal release processes are generally more effective at low than at high frequencies (Arya & Gulati 1968; Gillespie & Tilmisany 1976; Kirpekar et al 1977). A multitude of drugs are known to alter transmitter release and to compile from this an array of specific functional receptor systems regulating neurotransmitter release under ordinary conditions of neuronal excitation seems at present premature.

A reduction in the stimulation-induced efflux of ^{3}H-transmitter during the presence of exogenous noradrenaline has often been observed (McCulloch et al 1972; Starke 1972; Stjärne & Gripe 1973; Hedqvist 1974; Stjärne 1974; Enero & Langer 1975; Starke et al 1975; Hope et al 1976) but generally this effect has been scrutinized only at a single test frequency in any given experimental protocol. Similarly, the promotion of transmitter efflux by phenoxybenzamine has, in most cases, been studied only at one moderate frequency and additionally one inordinately high one, but always with results seemingly at variance with a system supposedly monitoring and responding to the environmental level of active transmitter.

It has been reported (Bell & Vogt 1971) that phenoxy-

benzamine enhanced output in a guinea-pig artery at 5 Hz but not at 25 Hz and a similar relationship was reported by others with 2 and 50 Hz in rat vas deferens (Vizi et al 1973), and with 5 and 30 Hz (Langer et al 1975) and 10 and 30 Hz (Brown & Gillespie 1957; Kirpekar & Cervoni 1963) in cat spleen. This ineffectiveness at very high frequencies was attributed by some to a disengagement of the feedback system (Langer 1977). However, another worker (Hughes 1972), who at the time assigned the effect of phenoxybenzamine on efflux entirely to block of inactivation processes, found that phenoxybenzamine enhanced efflux in the rabbit vas deferens to a comparable extent when 240 pulses was administered at any of the more physiologically probable frequencies of 2, 6 and 16 Hz; an observation not in keeping with an operative autoinhibitory system.

Other recent work has also questioned the validity of the hypothesis which assesses the effects of agonists and antagonists on neurotransmitter release exclusively in terms of a neuronal system routinely regulating release. Phenoxybenzamine elevated both the efflux of tritium and the mechanical response in reply to a single pulse delivered to the guinea-pig vas deferens, a situation which seemingly precludes the intervention of a negative feedback process (Kalsner 1979a). Also, the pattern of effect of phenoxybenzamine on transmitter efflux in both cattle renal artery and guinea-pig vas deferens, with changes in frequency, was not in accord with blockade of an autoinhibitory system (Chan & Kalsner 1979; Kalsner 1979b). Finally, a broad and intensive examination of a wide class of adrenergic antagonists showed that their effect on stimulation-induced transmitter efflux does not meet the usually accepted standard for a system designated α-adrenergic (Kalsner & Chan 1979). Noradrenaline and phenoxybenzamine clearly exert presynaptic actions to decrease and enhance transmitter output but the unitary hypothesis which assigns these effects to interactions with a functional autoinhibitory system mediated by adrenergic receptors should be reconsidered.

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REFERENCES

Arya, P. C., Gulati, O. D. (1968) Br. J. Pharmacol. Chemother. 33: 413–425

Bell, C., Vogt, M. (1971) J. Physiol. (London) 215: 509–520

Brown, G. L., Gillespie, J. S. (1957) Ibid. 138: 81–102

Chan, C. C., Kalsner, S. (1979) Br. J. Pharmacol. 67: 401–407

Enero, M. A., Langer, S. Z. (1975) Naunyn-Schmiedeberg's Arch. Pharmacol. 289: 179–203

Gillespie, J. S., Tilmisany, A. K. (1976) Br. J. Pharmacol. 58: 47–55

Hedqvist, P. (1974) Acta. Physiol. Scand. 90: 158–165

Hope, W., Law, M., McCulloch, M. W., Rand, M. J., Story, D. F. (1976) Clin. Exp. Pharmacol. Physiol. 3: 15–28

Hughes, J. (1972) Br. J. Pharmacol. 44: 472–491

Kalsner, S. (1979a) Ibid. 66: 343–349

Kalsner, S. (1979b) Can. J. Physiol. Pharmacol. 57: 717–724

Kalsner, S., Chan, C. C. (1979) J. Pharmacol. Exp. Ther. 211: 257–264

Kirpekar, S. M., Cervoni, P. (1963) Ibid. 142: 59–70

Kirpekar, M., Kirpekar, S. M., Prat, J. C. (1977) J. Physiol. (London) 272: 517–518

Langer, S. Z. (1977) Br. J. Pharmacol. 60: 481–497

Langer, S. Z., Dubocovich, M. L., Celuch, S. M. (1975) in: Almgren, O., Carlsson, A., Engel, J. (eds) Chemical Tools in Catecholamine Research. Vol. II. North Holland Publishing Co., Amsterdam, pp 183–191

McCulloch, M. W., Rand, M. J., Story, D. F. (1972) Br. J. Pharmacol. 46: 523–524P

Rand, M. J., McCulloch, M. W., Story, D. F. (1975) in: Davies, D. S., Reid, J. L. (eds) Central Actions of Drugs in Blood Pressure Regulation. University Park Press, Baltimore, pp 94–132

Starke, K. (1972) Naunyn-Schmiedeberg's Arch. Pharmacol. 275: 11–23

Starke, K. (1977) Rev. Physiol. Biochem. Pharmacol. 77: 1–124

Starke, K., Endo, T. (1976) Gen. Pharmacol. 7: 307–312

Starke, K., Endo, T., Taube, H. D. (1975) Naunyn-Schmiedeberg's Arch. Pharmacol. 291: 55–78

Stjärne, L. (1974) Acta. Physiol. Scand. 90: 286–288

Stjärne, L., Gripe, K. (1973) Naunyn-Schmiedeberg's Arch. Pharmacol. 280: 441–446

Vizi, E. S., Somogyi, G. T., Hadhazy, P., Knoll, J. (1973) Ibid. 280: 79–91