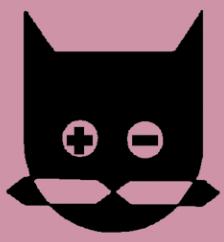

ADVANCES IN PHARMACOLOGICAL RESEARCH AND PRACTICE

General Editors: **J. KNOLL** and **K. KELEMEN**

Volume 2

RECEPTORS AND CENTRALLY ACTING DRUGS

Editors

E. S. Vizi, S. Fürst and **G. Zsilla**

PHARMACOKINETICS AND DRUG METABOLISM

Editors

K. Magyar, T. Szűts and **L. Vereczkey**

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Proceedings of the 4th Congress of the Hungarian Pharmacological Society, Budapest, 1985

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PREFACE

The Congresses of the Hungarian Pharmacological Society have become traditional meetings of pharmacologists from all over the world. At the 4th Congress, in 1985, six highly topical subjects were discussed in six symposia, by prominent scientists representing the pharmacological science in different continents of the world. The present volumes contain the full text of the invited lectures of the symposia giving a comprehensive view of the state of the art in the fields of Pharmacological Protection of the Myocardium; Pharmacology of the Vascular System; Receptors and Centrally Acting Drugs; Pharmacokinetics and Drug Metabolism; Dopamine, Ageing and Diseases; Endogenous Anorectics; Prostanoids. The vast amount of new information contained in the volumes are indispensable for experts working in these fields.

J. Knoll and K. Kelemen

General Editors

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Section 3

RECEPTORS AND CENTRALLY ACTING DRUGS

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TRANSMISSION AND RECEPTORS

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Sect. 3. Receptors and
Centrally Acting Drugs
E. S. Vizi, S. Fürst, G. Zsilla (eds)

ON THE SCOPE AND MECHANIÓSMS OF LOCAL CONTROL OF NEUROTRANSMITTER SECRETION FROM INDIVIDUAL VARICOSITIES OF THE SYMPATHETIC NERVES OF THE GUINEA-PIG AND MOUSE VAS DEFERENS

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INTRODUCTION

The recent discovery that (many) sympathetic nerves do not utilize only noradrenaline (NA) but also for example adenosine 5'triphosphate (ATP)[20,21] and neuropeptide Y (NPY)[13] as transmitter substances has important consequences for our understanding of sympathetic neurotransmission and its prejunctional control (Fig. 1) . The relative prejunctional importance of each of these putative cotransmitters will not be discussed in detail in the present paper, nor that of other signal substances ('X', 'Y' and 'Z' in Fig. 1), derived from effector cells, from other nerves or from the circulation. The paper concentrates instead on the scope of prejunctional regulation of neuromuscular transmission (i.e. the extent to which the various control systems modulate the secretory response of the terminals to nerve impulses in the parent axon), and the mechanisms whereby such control is exerted. By a novel electrophysiological method[3] it seems now possible to analyse how the local control of transmitter secretion in the sympathetic nerve terminals of the guinea-pig and mouse vas deferens operates at the level of the individual varicosity.[6] The conclusions concerning the scope and mechanisms of prejunctional control are therefore expressed, tentatively, in terms of induced changes in the number

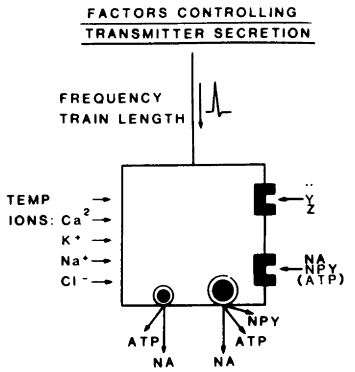

Fig. 1. Diagram illustrating different factors which influence the basic mechanisms, and the local modulation, of transmitter secretion from sympathetic nerve terminals. For explanation see the text.

of varicosities activated ('N'), and/or in the probability ('P') or quantal content ('Q') of transmitter secretion from individual varicosities.[25,29] Due to the limited space available, mainly work from my own laboratory is quoted; in these original papers the reader will find pertinent references to the literature.

SCOPE OF LOCAL CONTROL OF SECRETORY ACTIVITY OF INDIVIDUAL VARICOSITIES

A. A model based on biochemical and electrophysiological evidence: The bulk of the NA in the varicosity is stored in vesicles; in the sympathetic nerves of rodents vas deferens the average varicosity contains some 500 transmitter vesicles. If each impulse activates every varicosity, releasing one quantum of transmitter (according to the vesicle hypothesis, the contents of a vesicle), then each suprathrehold nerve stimulus should release 1/500 of the tissue content of NA into the extraneuronal space. But in all tissues examined (even after blocking the reuptake of NA) the observed 'overflow' of NA per stimulus is two orders of magnitude smaller, about 1/50000 of the tissue content (see Figs. 2,3). Two mutually exclusive hypotheses have been advanced to explain the strikingly parsimonious behaviour of the secretory mechnisms: 1. In the non-intermittency model[10] it was assumed on on other grounds, that every nerve impulse activates every varicosity. Therefore from the abovementioned results it was concluded that the NA quantum is only a small fraction (about 1%) of the contents of a vesicle (Q =0.01). 2. In the intermittency model[2] the vesicle hypothesis (that it is the contents of a vesicle which constitutes the transmitter quantum) was assumed to apply (Q = 1). From that it was concluded that each nerve impulse activates only a small proportion (according to later estimates[25], about 1%) of the varicosities (P = 0.01). The choice between these alternatives required that either P or Q could be measured directly. At least in the sympathetic nerves of the guinea-pig and the mouse vas deferens, this now seems possible, by an electrophysiological method,[3] which may permit study of the secretory activity of individual varicosities on an impulse by impulse basis.[6] Perhaps for technical reasons (differences in resolution) interpretation of evidence obtained by this method has not been incontroversial.[3,4,6] In my laboratory, results obtained under more optimal conditions strongly support the 'intermittency' hypothesis, and suggest that the secretion of transmitter from individual varicosities of the sympathetic nerves of the guinea-pig and mouse vas deferens has the followng characteristics:[6] During low frequency stimulation nerve impulses in the parent axon only infrequently activate the secretory mechanisms of the average individual varicosity (P=0.002-0.03); whenever activated the varicosity secretes only a single quantum (Q = 1). This extreme secretory intermittency is due in part to failure of most nerve impulses to invade the varicosity, but in part also to restrictive mechanisms in the varicosity itself. Nerve impulses do not seem to induce secretion from randomly located vesicles but only from those (2 - 3) which are positioned at 'preferred release sites', from which quanta may be secreted in 'complementary pairs'. Release of the first quantum of a 'pair' does not induce autoinhibition of that release site, but the opposite: shortlasting facilitation. Release of the second quantum is followed by long secretory silence in that site.[6] It should be noted that the transmitter whose quantal secretion is measured by the electrophysiological method is not NA but ATP.[27]

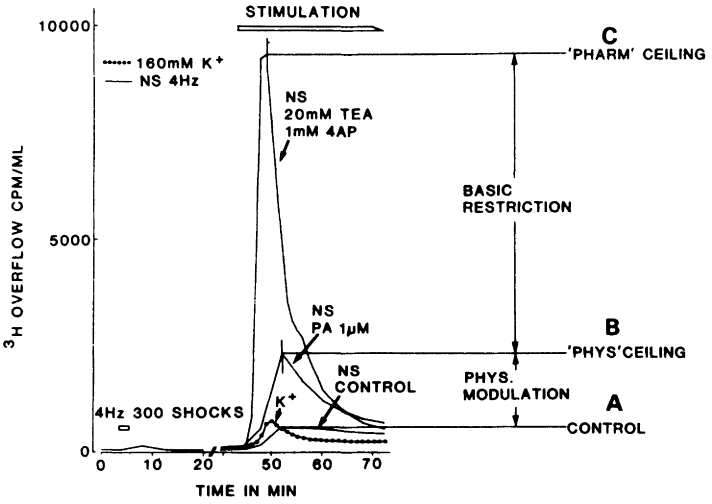

Fig. 2. The stimulus-induced 'overflow' of ^{3}H-NA (as reflected in the increase in efflux of ^{3}H) in the guinea-pig vas deferens, in which the NA stores of the sympathetic nerves had been labelled by preincubation with ^{3}H-NA.[1] Effects of addition of phentolamine to block α_2-adrenoceptor mediated autoinhibition, or of tetraethylammonium (TEA) and 4-aminopyridine (4AP) to block K^+ and/or promote Ca^{2+} conductances,[15] on the secretory response to continuous electrical nerve stimulation ('NS') at 4 Hz. For comparison, the secretory response to direct depolarization of varicosities by high K^+ is also shown. Means and range of 2 experiments. For the significance of levels A, B and C, see the text.

The electrophysiological method does not permit observations during stimulation at high frequency (since contraction of the preparation dislodges the micro-electrode). But at least within the frequency range 0.5 - 2 Hz there is some experimental evidence that the quantal secretion of ATP (as measured by this method)[6] may reflect indirectly the secretion of NA also.[6] NA and ATP may therefore be secreted in parallel as components of mixed sympathetic transmitter quanta.[27] It will have to be decided in future work precisely how well the model applies in detail to the quantal (?) secretion of NA. Meanwhile in the present paper it is assumed to be valid in principle.

B. The maximal steady state secretory rate: In the absence of drugs increasing transmitter secretion, the level of the 'overflow' of ^{3}H-NA from the sympathetic nerves of the guinea-pig vas deferens evoked by continuous nerve stimulation could be maintained only at frequencies up to 4 Hz (level A in Fig. 2). At higher frequencies the output initially was higher but showed rapid fading. At level A the stimulus-evoked 'overflow' represented secretion of 0.5% of the tissue store of ^{3}H-NA each minute, or an output from the average varicosity (containing 500 vesicles) corresponding to the transmitter contents

of ((0.5 x 500)/100 =) 2.5 vesicles/min. Even in the presence of drugs initially increasing the output, fading during continued nerve stimulation caused it gradually to approach level A (Fig. 2). The exocytotic[17] secretory response to complete depolarization of all varicosities by 160 mM K^+ was also in this range (Fig. 2). These findings suggest 1. That level A may represent the maximal steady state secretory ratef these nerves, 2. That this level is 'set' by intrinsic properties of the varicosities, not subject to physiological or pharmacological modulation (?), and 3. That the rate limiting step may be the time (apparently 20 - 30 seconds) it may take under steady state conditions, to recharge emptied 'preferred release sites'.[6]

C. The 'physiological' maximum of the peak secretory rate: Phentolamine (added to block prejunctional α_2-adrenoceptors) increased the peak secretory response by about 5-fold (Fig. 2, 3). Results in other experiments indicate that inhibition of the secretory mechanisms of these nerves by the sympathetic cotransmitters ATP or NPY (at least at low nerve impulse frequency), or by other signals ('X', 'Y' or 'Z' in Fig. 1; i.e. agents such as adenosine or endogenous prostaglandins) is quantitatively less important than that mediated by NA.[24,28,30] The initial peak level in the presence of phentolamine (B in Fig. 2) therefore may represent that when the secretory mechanims of the nerves are almost completely liberated from 'autoinhibition', i.e. the 'physiological ceiling' of the secretory response of the terminals to impulses in the parent axon.[25,29] Thus the difference between levels B and A in Fig. 2 may represent an approximation of the scope of the 'physiological' modulation of transmitter secretion. The secretory activity of the individual varicosities seems to remain intermittent and monoquantal in the presence of α_2-blocking agents (Cunnane and Stjärne, unpublished). Presumably therefore, liberation of the secretory mechanisms from 'α-autoinhibition' increases transmitter secretion mainly by recruiting earlier inactive varicosities (i.e. in electrophysiological terms, by increasing 'N', rather than 'P', without changing 'Q'). It appears therefore that 'α-autoinhibition' is a very minor cause of the secretory intermittency of the individual varicosities, merely deciding if each nerve impulse in the parent axon will activate 5%, or down to only 1% of the 'monoquantal' varicosities.

C. The 'pharmacological' maximum of the peak secretory rate: Level B in Fig. 2 does not represent the absolute secretory maximum of these nerves; the output could be strongly enhanced by certain pharmacological agents, such as tetraethylammonium (TEA) and 4-aminopyridine (4AP), which do not act on prejunctional receptors. The initial peak output on stimulation at 4 Hz in the presence of these agents (level C in Fig. 2) could not be increased further by increasing the stimulus frequency, however, and may therefore represent the maximal secretory rate in these nerves, i.e. their 'pharmacological secretory ceiling'.[25,29] Now each minute the nerves secreted about 8% of the tissue content of ^{3}H-NA,[29] and hence transiently the average varicosity released an amount corresponding to the transmitter contents of ((8 x 500)/100=) 40 vesicles/min. On continuous stimulation at 0.25 - 16 Hz in the presence of TEA and 4AP the initial peak secretory response was always followed by rapid decline; to maintain a stable output it was necessary to reduce the stimulus frequency to about 0.06

Hz (time interval between successive shocks: 16 sec).[25,29] Under these conditions each stimulus released about 1/500 of the tissue store of ^{3}H-NA, corresponding to the contents of one vesicle from each varicosity. Thus transiently the varicosity can secrete as many as 40, but under steady state conditions not more than about 3 quanta per min. The minimum time interval between nerve stimuli (about 20 seconds) required to maintain steady state secretion in a varicosity was the same, regardless of blockade of K^+ efflux or promotion of Ca^{2+} influx (by TEA + 4AP). Evidently therefore these ionic mechanisms do not affect the step which is rate limiting under these conditions (steady state recharging of emptied 'preferred release sites'?).

D. A basic 'economy' system: From the evidence discussed above it appears that inhibition of the secretory mechanisms of these sympathetic nerves mediated via prejunctional receptors accounts only to a minor extent for the 'normally' restricted secretory response (represented by the difference between C and A, Fig. 2) to nerve impulses in the parent axon. The main restriction (C - B) seems to be due to built-in mechanisms not subject to physiological modulation. The function of this system may be to protect the transmitter stores (only 500 quanta of transmitter in the individual varicosities), thus helping to maintain the transmission capability of the nerves.

LEVELS OF LOCAL CONTROL OF TRANSMITTER SECRETION IN SYMPATHETIC NERVES

The reason why transmitter secretion from a varicosity is monoquantal is not known. Nerve impulses seem to release transmitter only from the 'preferred release sites' of the varicosities; apparently these sites are unable to secrete more than one quantum per nerve impulse.[6] The high degree of secretory intermittency of the individual varicosities may be due in part to frequent failure of nerve impulses effectively to depolarize all varicosities, but in part also to intermittent and transient (post-secretory) refractoriness of the secretory mechanisms of the varicosities, to depolarization.[6] This in turn may reflect the existence of rate limiting steps in the turnover of vesicles at 'preferred release sites', the transient maximal or steady state rates of which may be 40 or 3 vesicles/min, respectively, to judge from the evidence above. Presumably the physiological control of transmitter secretion involves action at two levels: both on the invasion of the nerve terminals, and on the efficiency of depolarization-secretion coupling in invaded varicosities.

TEA and 4AP are known to block voltage dependent K^+ influx, and both directly (by effects of 4AP on Ca^{2+} channels) and indirectly (by prolongation of the duration of the nerve action potentials) to promote Ca^{2+} influx into nerve terminals.[15] TEA and 4AP increase transmitter secretion evoked by electrical nerve stimulation (which is tetrodotoxin-sensitive, and hence requires impulse propagation to the terminals by 'Na^+ action potentials'), but not that caused by high K^+,[29] (which is tetrodotoxin-resistant and hence due to direct depolarization of varicosities).[1] Presumably therefore TEA and 4AP increase transmitter secretion by promoting the invasion of nerve terminals. In that case it follows that nerve impulses in the parent axon do not normally fully invade all nerve branches. The fin-

dings with TEA and 4AP suggest that the reason for such intraterminal conduction failure is a built-in 'leakiness' of the nerve membrane to K^+, and restriction of Ca^{2+} influx; these features may be increasingly pronounced in 'distal' regions of the nerve branches.[7] Due to these built-in 'faults', transmitter secretion would be prevented from rising above a 'physiological ceiling' because each individual string of secretory varicosities is invaded by the nerve impulse only intermittently, and on a rotational basis.[25,29]

For the local 'physiological' fine-tuning of transmitter secretion, there exist in principle four different possible levels of action: 1. In some nerves (e.g. those of squid giant synapse[9]) large enough to permit impalement with intracellular micro-electrodes both pre- and postsynaptically, it has been shown that the presynaptic nerve impulse propagates actively to the terminal tip, and that the evoked secretion of transmitter (as judged by the amplitude of the evoked postsynaptic potential) is directly proportional to the amplitude and duration of the presynaptic nerve action potential.[9] In these nerves agents depressing transmitter secretion may act by lowering the presynaptic resting membrane potential, thus reducing the amplitude of the presynaptic action potential.[12] 2. In other nerves, with a low safety factor for active invasion of the secretory terminals, agents depressing transmitter secretion may do so by inducing presynaptic hyperpolarization, thus increasing the probability of failure of the nerve impulse to invade the secretory terminals.[12] 3. In nerves whose terminals are passively invaded, agents depressing transmitter secretion may act by increasing the conductance of the membrane of the nerve terminals, thus reducing their space constant and thereby the degree of depolarization (and secretory response[18]) of each consecutive varicosity of the terminal branches.[15,25] 4. Finally, agents depressing transmitter secretion may act by depressing the secretory responsiveness of the varicosity, to depolarization.[1,19,22,24] In the sympathetic nerves of the guinea-pig and mouse vas deferens, mechanisms 2 - 4 are likely to be involved. As suggested in the diagram in Fig. 1, numerous other factors are known to influence and/or mediate the prejunctional control of transmitter secretion from sympathetic nerves. Due to the limited space available, these aspects will be only briefly discussed here.

MECHANISMS OF PREJUNCTIONAL CONTROL

A. Influence of the nerve impulse pattern: The frequency and/or train length of nerve impulses has two important modulatory effects: 1. To induce facilitation, i.e. increase the amount of transmitter secreted per nerve impulse. This is a well-known if not well understood aspect;[1,22] it will not be discussed further here. 2. To influence the choice of transmitter secreted. As shown in pig spleen, an increase in the frequency and train length of stimulation of sympathetic nerves increases the secretion of NPY more than that of NA.[14] This may imply that nerve impulses at low frequency trigger secretion of transmitter (NA + ATP) mainly from 'small dense cored' vesicles, while during bursts at high frequency the nerve impulse preferentially activates secretion of transmitter (NPY + NA + ATP) from 'large dense cored' vesicles.[11,14,30,32]

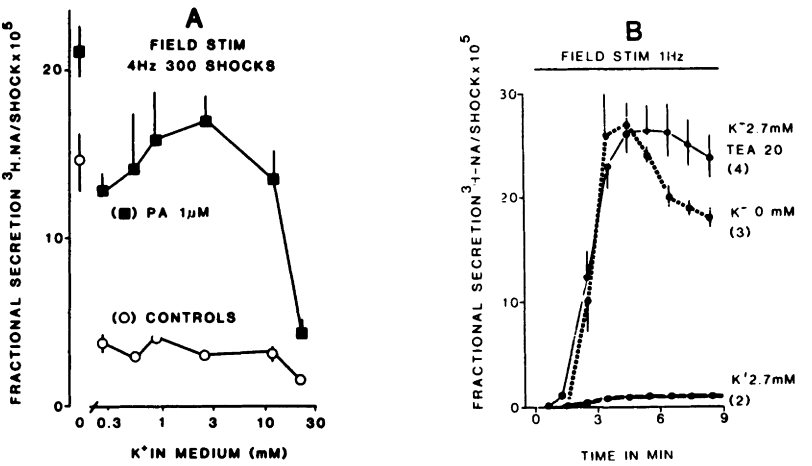

Fig. 3. Influence of K^+ in the medium on the secretory response of the sympathetic nerves of the guinea-pig vas deferens to electrical nerve stimulation. (A). Responses to nerve stimulation with trains of 300 shocks at 4 Hz, as a function of the K^+ concentration in the medium, in controls and after addition of phentolamine (PA) to block 'α -autoinhibition'. Means + SE (n = 4 - 12). (B). Responses to continuous nerve stimulation at 1 Hz at 2.7 mM K^+ in controls and in the presence of tetra-ethylammonium (TEA); effect of 0 K^+. Means and range (when n=2), or means + SE (n = 3 - 4).

B. Influence of temperature: In all nerves examined, the spontaneous secretion of transmitter quanta increases steeply with temperature but moderate cooling from 36°C did not reduce K^+-evoked secretion of NA from sympathetic nerves in rabbit heart[17] or guinea-pig vas deferens[26] (and in vas deferens in fact increased the secretory response to electrical stimulation[26]). Thus neither the exocytotic[17] event per se nor depolarization-secretion coupling seem to be energy-requiring, or enzymatically mediated;[17,26]. Energy may be required instead to restrict transmitter secretion.

C. Roles of Ca^{2+}: Transmitter secretion requires Ca^{2+} in the medium; the secretory response to electrical nerve stimulation increases, and the efficiency of 'α-autoinhibition' decreases, with the extracellular Ca^{2+} level[1,5,19,24]. Activation of prejunctional 'autoinhibitory' receptors thus seems somehow to make external Ca^{2+} less available for the secretory mechanisms. Little is known about precisely how and where. The effect may be to reduce the efficiency of depolarization-secretion coupling in invaded varicosities,[19] but in part also to depress Ca^{2+}-dependent step(s) involved in the conduction of nerve impulses to the varicosities.[1,7,22,24]

D. Roles of K^+: As shown in Fig. 3A, both the control level of the secretory response of the sympathetic nerves of the guinea-pig vas deferens to electrical nerve stimulation, and the increased level after blockade of 'α-autoinhibition', were remarkably independent of changes in the K^+ concentration in the medium, in the range 0.2 - 10 mM.

Hence transmitter secretion in these nerves is surprisingly[9] uninfluenced by changes in the resting membrane potential. Possibly therefore the secretory parts of these nerve terminal are not invaded actively, but passively and decrementally.[24] Removal of K^+ from the medium increased the secretory response in controls by 5-fold; in the presence of an α-blocking agent the relative effect of 0 K^+ was smaller. The effect of 0 K^+ was not mimicked by ouabaine at up to 10^{-4}M, but matched almost exactly by addition of TEA[8] (Fig. 3B); it may be due to blockade of voltage dependent K^+ channels. The results show that 'α-autoinhibition' in these nerve terminals is not much influenced by changes in the prejunctional resting potential; it may operate by increasing the K^+ conductance,[16] reducing the degree of depolarization of the more distal varicosities.[15]

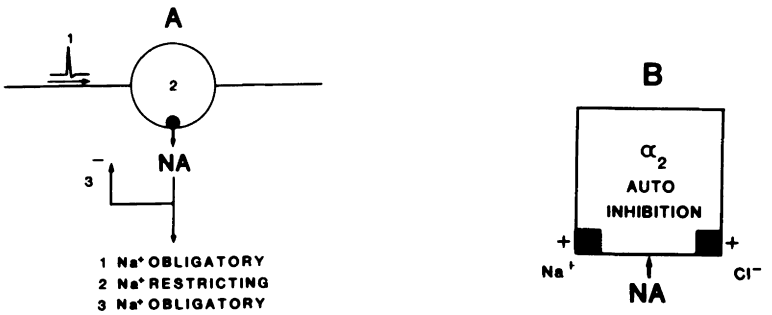

Fig. 4 (A). Three different levels where Na^+ in the medium influences stimulus-secretion coupling in the sympathetic nerves of the guinea-pig vas deferens.[23] (B). The presence of Na^+ in the medium is obligatory for 'α-autoinhibition', and Cl- must be present for its full expression.[31] For comments see the text.

E. Roles of Na^+: Sodium ions may have three roles in stimulus-secretion coupling in the sympathetic nerves of the guinea-pig vas deferens: 1. Impulse conduction to the terminal regions of these axons requires the presence of Na^+ in the medium, and is blocked by tetrodotoxin.[7] 2. Depolarization-secretion coupling in the varicosities seems to be restricted by Na^+. 3. The presence of Na^+ in the medium is obligatory for 'α-autoinhibition' (Fig. 4B).[23]

F. Role of Cl^-: The presence of Cl^- ions in the medium seems to be required for full expression of 'α-autoinhibition' of the secretory response of the sympathetic nerves of the guinea-pig vas deferens, to electrical nerve stimulation (Fig. 4B). Presumably Cl^- ions exert their effect on the external aspect of the nerve membrane.[31]

TENTATIVE CONCLUSIONS

The varicosities of these nerves cannot secrete more than one ('mixed') transmitter quantum at a time. Numerous factors contribute to the complex local control of the probability (P) that a nerve impulse in the parent axon will activate the individual varicosity. The scope of such control seems to be limited to the range (P = 0.002 - 0.1).

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