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Neuromuscular transmission in arterioles

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Introduction

It is often assumed that superfusion of a smooth muscle organ with the putative transmitter, released by the nerves which innervate that organ, will produce a response identical to that produced by nerve stimulation. Many observations have been made which are in accord with this view. As simple examples, sympathetic nerve stimulation and superfusion with noradrenaline, the presumptive transmitter, each produce vasoconstriction in most vascular beds, frequently each of these mechanical responses are abolished by the same antagonist. Secondly, the sympathetic nerves which innervate many smooth muscle organs do not give rise to specialized

synaptic structures, rather they form a diffuse network of varicose fibers. Varicosities, the presumptive sites of transmitter release, often lie up to, and occasionally more, than a micron from the membrane of the smooth muscle cells making up that organ[2]. Such an arrangement could reasonably be expected, like superfusion, to load the extracellular space with transmitter. Indeed the earlier electrophysiological analyses of neuromuscular transmission at smooth muscle organs supported such a view; the membrane potential changes which resulted from nerve stimulation could be modelled only with the assumption that transmitter diffused from its points of release and persisted in the extracellular space for extended periods (for review, see Holman and Hirst[20]). However a number of more recent electrophysiological observations argue against the idea that neural control is exerted in such a 'loose' manner, rather they suggest that control is exerted in an ordered manner not unlike that described for the skeletal neuromuscular junction[10] and for central nervous system synapses[32]. This article aims to summarize the arguments for the latter view with special reference to sympathetic transmission in arterioles. Subsequently the possibility that, if the primary transmitter released by sympathetic nerves innervating systemic arteries is noradrenaline, the functional neural contacts between sympathetic nerves and arterial smooth muscle cells have specialized junctional receptors: (γ-receptors), will be explored.

Most arteries and arterioles are wrapped by sympathetic nerve fibers which, after exposure to formaldehyde when viewed with ultraviolet illumination, show a characteristic fluorescence[29]. The evidence that such nerves store, synthesize and release noradrenaline has been reviewed[11]. Individual axons usually do not penetrate the muscular wall of either arteries or arterioles, rather they ramify on the adventitial surface. Where distinguishable, single axons running across the surface of an artery have a varicose appearance, the individual varicosities being separated by some 10 µm lengths of fine unmyelinated axon. Similarly the nerve fiber giving rise to the nerve terminals is often varicose[2]. Studies using electron microscopy indicate varicosities contain vesicles, yet have failed to demonstrate the other pre- or post-synaptic specializations which are normally associated with conventional synaptic contacts. Moreover there is a considerable variation in the separation between varicosities and the nearest smooth muscle membrane both between different arteries and between successive varicosities of individual arteries[2]. The smooth muscle cells which make up the muscular wall of an artery or arteriole are individually arranged with their long axes in a circumferential orientation. Individual smooth muscle cells have diameters at their thickest part of about 4 µm and lengths of about 60 µm (see, for example, Hua and Cragg[22]). Areas of contact between individual smooth muscle cells have been detected in electron micrographs; these are thought to be the structural units which permit electrical coupling between neighboring smooth muscle cells[2].

Biophysical aspects of transmission in arterioles

In every artery examined, as with all other smooth muscle organs, electrophysiological studies indicate that individual smooth muscle cells are electrically connected to their neighboring cells to form syncytia[21,28]. This has been shown by subjecting a portion of an artery to a voltage field, so inducing transmembrane current flow in that portion[21]. Since membrane potential changes can be detected away from the voltage field, the induced current flow must be passively spreading down the artery. Clearly low resistance pathways must exist between the cells in the voltage field and those away from it. A consequence of the syncitial nature of arterial smooth muscle is that when junctional current flows across the membrane of a smooth muscle cell on the adventitial surface, that current will lead to charge displacement on the membranes of the smooth muscles even making up the luminal surface. Whether or not endothelial cells of the lumen are coupled to smooth muscle layers is not known. Electrical coupling between arteriolar smooth muscle cells has also been demonstrated by making paired intracellular recordings from smooth muscle cells separated but within the same arteriolar tree. When current was injected via one electrode a membrane potential change was detected at the second recording point. Current must flow between the individual smooth muscle cells, a proportion leaking across the membrane resistance of each and so producing a voltage change[15].

Perivascular nerve stimuli applied to all arteries and arterioles examined to date initiates excitatory junction potentials (e.j.ps) in the smooth muscle layer[1,12,13,21,27,33]. With the exception of pulmonary arteries (and most veins) which have distinctly slow e.j.ps[34], e.j.ps recorded from a variety of species and vascular beds are similar. E.j.ps have times to peak depolarization of 50–100 msec; after the peak potential the e.j.ps decay with a time course which can be described by a single exponential (time constant of decay 100–700 msec). In arterioles of guinea pig submucosa the membrane time constant of the smooth muscle cells is the same as the time constant decay of the e.j.p.[15]. Similar observations have been made on the rabbit saphenous artery[21]. These findings indicate that the duration of excitatory current flow responsible for an e.j.p. is brief when compared to the time course of that e.j.p.[7,15,25].

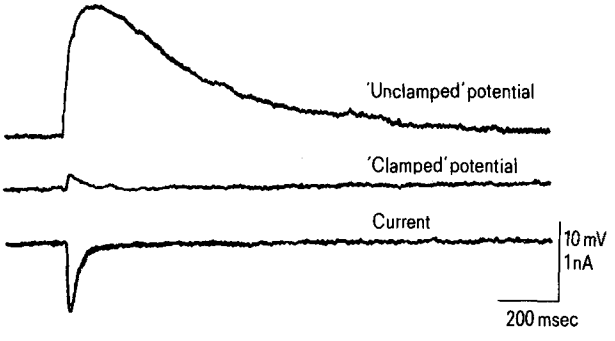

Figure 1. Comparison between the time courses of an excitatory junction potential and its underlying excitatory junctional current. Recordings from short segment of submucosal arteriole of guinea pig. It can be seen that the duration of current flow is brief when compared with that of the 'unclamped' e.j.p. Note also the small peak amplitude of the excitatory junctional current. Each record is the average of 20 successive stimuli.

A more direct measure of the time course of transmitter action, or more correctly the time course of excitatory junctional currents (e.j.cs) initiated by transmitter action, can be obtained using a voltage clamp technique[8]. An example of an e.j.p. and the e.j.c.underlying it is shown in figure 1. It can be seen that the peak current occurs within 10 msec and that the current is essentially finished by 150 msec. The decay of the currents can again be described by single exponentials, the time constants of decay being in the range 40–60 msec[8]. E.j.c. amplitude, membrane potential relationships indicate that the e.j.c. has a null potential close to 0 mV. Qualitatively similar observations have been made at the skeletal neuromuscular junction[36] and at a central synapse[9,24]. It is of interest to note that a similar model, i.e. a relatively brief transmitter action, has been proposed for transmission in the vas deferens; it appears that the earlier analyses of transmission erred in that the choice of membrane time constant was inappropriate (for further details see Bywater and Taylor[4]).

Despite the structural evidence that arteriolar smooth muscle receives a dense innervation, electrophysiological studies indicate that during neuromuscular transmission only a few synapses release quanta per nerve impulse[16]. Thus, at rest spontaneous excitatory junction potentials are detected; these are generally considered to result from the spontaneous release of quanta of transmitter[25]. When a large population of varicosities are stimulated the resultant e.j.p. consists of only a few quanta[16]. Similar suggestions have been made for transmission in the vas deferens[3,20]. This means that transmitter is likely to be released at only a few points, these being spatially separated from each other. In a structure such as an arteriole, the wall of which consists of a monolayer of smooth muscle cells, it is hard to see how transmitter released at only a few points would either activate a large proportion of arteriolar muscle membrane area or remain for a prolonged period in contact with those membranes. A problem as yet unresolved is whether the low quantal content of e.j.ps reflects the fact that all varicosities can release transmitter but do so with a very low probability of release or whether only a proportion of varicosities make functional synaptic contacts. The observation that action potentials passing in an antidromic direction in pre-terminal axons fail to initiate 'local' e.j.ps indicates that functional contacts along this area of sympathetic nerve do not exist[12].

When the amplitudes of junctional currents caused by the spontaneous release of quanta of sympathetic transmitter were measured, they were found to have low peak intensities (0.2 nA[8]). The amplitude of the current resulting from the activiation of a single sympathetic receptor channel is not known. However, at most synapses with a variety of transmitters, single channels activated at normal resting potential produce a current of about 1 pA. If such a value was appropriate for arteriolar neuromuscular junctions, the number of receptor channels activated by a quantum of transmitter would be relatively small and it may be that the amplitude of a single response is restricted by the number of receptors available for activation. Similar suggestions have been made for autonomic ganglia[14,31] and for a central synapse[23].

Receptor specialization

The preceding sections have dealt, briefly, with some of the cellular aspects of sympathetic nerve transmission in arterioles. They have suggested that transmission results from the synchronous release of a number of pre-formed packets of transmitter; however the number of packets released per impulse per length of arteriole is far smaller than the number of histologically detected sympathetic nerve varicosities per length of arteriole[16]. The junctional currents which underlie the e.j.p. are brief when compared with the time course of the e.j.p.; the long time course of the latter being attributable to the high membrane resistance of the arteriolar smooth muscle cells[15,16] rather than to a long lasting transmitter action. Sympathetic junctional currents are of small amplitude, this follows from the low quantal content of the evoked current and from the small amplitude of individual junctional currents[8]. The preceding sections, however, have not tackled the problem of the identity of the transmitter nor whether as at other synapses[26] areas of specialized junctional receptors exist near sympathetic nerve endings.

Whereas there are many reports which indicate that the majority of sympathetic post ganglionic axons are able to synthesize, store, release and inactivate the presumptive transmitter noradrenaline[2,11], attempts to mimic the action of sympathetic nerve stimulation by superfusion of arteries with noradrenaline have, at a cellular level, failed to generate clear support for a role of this substance as a transmitter. For example, noradrenaline applied by superfusion causes arterial constriction. However large increases in tension are generated which are associated with either a very small or undetectable change in membrane potential[5]; such contractile responses are readily abolished by α-adrenoreceptor antagonists[2]. In contrast, sympathetic nerve stimulation causes membrane depolarization (i.e. e.j.ps). The e.j.ps must sum to activate voltage dependent conductance changes in the arteriolar smooth muscle before constriction occurs[13,21]. Moreover e.j.ps in all systemic arteries reported to date, are unaffected by α-adrenoreceptor antagonists[7,27]. An explanation for this paradox, which would be in accord with the view that noradrenaline was the transmitter, could be that receptor specialization occurs, the receptors under the sympathetic nerve terminals having different pharmacological properties and ionic properties to those accessed by superfused noradrenaline (or circulating catecholamines). Some observations, in accord with this idea, have been made[18]. Noradrenaline, applied by ionophoresis, produces rapid membrane depolarizations only when applied near sympathetic nerve endings[18]. These responses are resistant to α-blockade. In contrast, noradrenaline, when applied to extrajunctional regions by ionophoresis produces constriction with little or no associated membrane change; such responses are abolished by α-antagonists[17]. These observations indicate that the noradrenaline receptors in the vicinity of the nerve terminal are not of the α-type and they have been termed γ-receptors[19]. In arteries such as the rat basilar artery, the pharmacological properties of such presumed junctional receptors, when tested with a restricted number of agonist, applied in high concentrations by superfusion, again had pharmacological properties distinct from α-or β-re-

ceptors[19]. Such observations imply that a specific antagonist for γ-receptors should exist; only when such a compound is found should the idea of specific junctional receptors find acceptance.

Action of prazosin on arterioles

In view of the inability of α-adrenoreceptors antagonists to reduce the amplitude of e.j.ps, it is perhaps surprising that a number of chemicals which block α-adrenoreceptors reduce the mechanical responses to sympathetic nerve stimulation. In some tissues the explanation is straightforward. For example, in pulmonary arteries (and veins), sympathetic nerve stimulation evokes membrane depolarizations which are slower than those recorded from systemic arteries (e.g. Suzuki[34]). Such arteries unlike systemic arteries (and veins) lack γ-receptors (J. Bevan, personal communication) and the sympathetically induced membrane potential changes reflect α-receptor activiation[34]. In rat tail arteries and rabbit ear arteries, thermoregulatory arteries, the responses to nerve stimulation are biphasic, the initial rapid components (e.j.ps) being α-resistance, the slower component resulting from α-activation[6,35]. However such explanations are not appropriate for systemic arteries where constriction appears to result entirely on activation of voltage-dependent conductance changes in the arteriole smooth muscle. The action of one agent, prazosin, which does reduce the mechanical responses to sympathetic nerve stimulation in systemic vessels has been examined on submucous arterioles. This compound is a potent selective α-antagonist on submucous arterioles yet in higher concentration acts in a non-specific manner. An experiment illustrating this is shown in figure 2. The diameter of an arteriole was monitored, before, during and after superfusion with a concentration of noradrenaline (1×10^{-6} M) which caused a submaximal constriction (fig. 2A). This response was reduced by the addition of prazosin (2×10^{-11} M) (fig. 2B). The response to 2×10^{-6} M noradrenaline in this same concentration of prazosin was approximately the same as that produced by 1×10^{-6} M noradrenaline in the absence of prazosin (fig. 2C). Evidently the affinity of prazosin for the adrenoreceptor activated by superfusion with noradrenaline is high. However such concentrations of prazosin had no effect on evoked e.j.ps recorded from the same arterioles. Moreover increasing the concentration of prazosin to 1×10^{-6} M had no effect on evoked e.j.ps (fig. 3A, B). The low concentrations of prazosin similarly had no effect on the mechanical responses generated by sympathetic nerve stimulation but higher concentrations had. An experiment is shown in figure 4A, B, C. Again the diameter of an arteriole was monitored during and after stimulation of the sympathetic nerves to that preparation with progressively increased numbers of stimuli (stimulation frequency 10 Hz). An example of a response is shown in figure 4A, it can be seen that these stimuli exoked a constriction and that this constriction is reduced by ex-

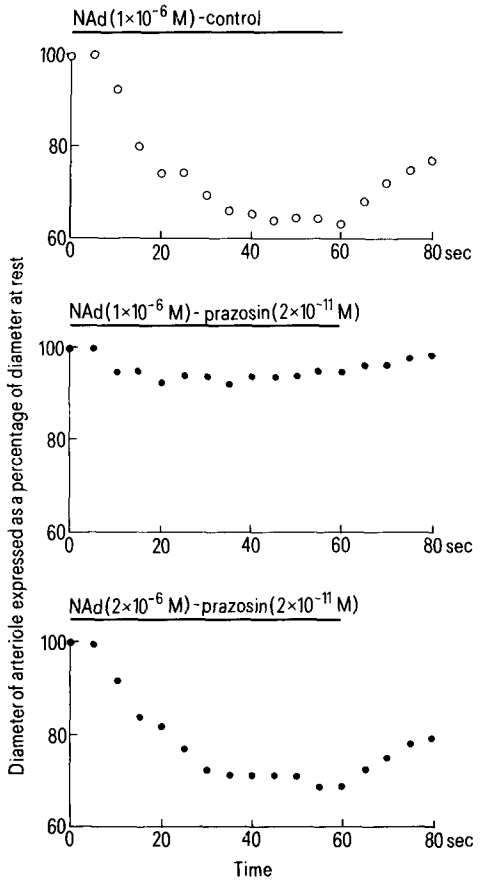

Figure 2. Ability of prazosin to block the vasoconstrictor effects of noradrenaline applied by superfusion to a guinea pig submucosal arteriole. Record *A* illustrates the time course of the change in diameter of an arteriole before, during and after superfusion of an arteriole with noradrenaline (1×10^{-6} M). Note the constriction is slight, reflecting the lower sensitivity of arterioles, where compared to arteries, to α-activation by noradrenaline *B* shows the response to this same concentration of noradrenaline (1×10^{-6} M) obtained in the presence of prazosin (2×10^{-11} M). This concentration had been present in the superfusing fluid for 10 min prior to this response. *C* illustrates the change of diameter of the arteriole in response to 2×10^{-6} M noradrenaline again in the presence of prazosin (2×10^{-11} M). Note that the response is very similar to that produced by 1×10^{-6} M noradrenaline in the absence of prazosin. All records obtained from same preparation by video recording image of arteriole.

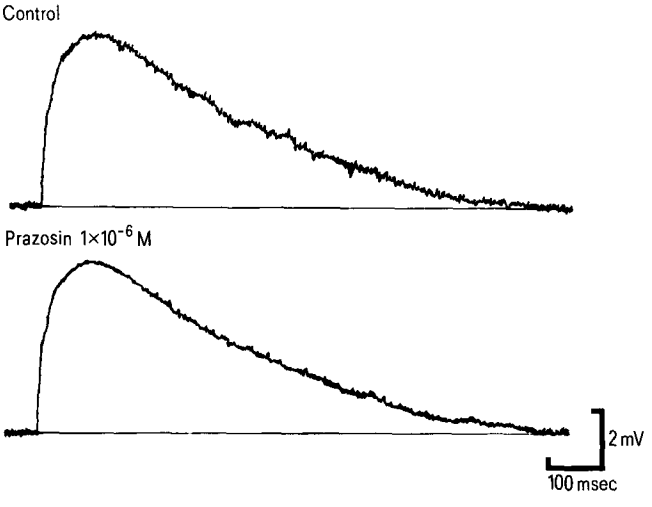

Figure 3. Lack of effect of prazosin (1×10^{-6} M) on e.j.ps recorded from guinea pig submucosal arteriole. In *A* a control response is shown, in *B* the response obtained 15 min after changing to prazosin (1×10^{-6} M). Each trace is the average of 10 successive responses, stimulation frequency 0.2 Hz.

posing the tissue to prazosin (1×10^{-6} M) (fig. 4B). When a stimulus/response curve was constructed in the absence and presence of prazosin it became apparent that more stimuli were required to initiate equivalent responses in the presence of prazosin (fig. 4C). During these experiments it was also noticed that this effect of prazosin was slower in onset than its ability to prevent noradrenaline constrictions, requiring some 10–20 min before consistent responses were obtained. Since the e.j.ps in this tissue were unaffected by prazosin, either noradrenaline could be 'leaking' from the noradrenergic synapses and causing α-activation or alternatively prazosin had some other direct depressant effect. The latter appeared to be correct. When constrictions were evoked by direct stimulation of the arteriole, these mechanical responses were 'inhibited' by prazosin (fig. 5). In these experiments the membrane potentials of short segments of arteriole were voltage clamped. The membrane potential was stepped from rest to a variety of depolarized potentials for 1 sec periods and the corresponding constrictions recorded. When this series of potential changes were repeated in the presence of prazosin larger membrane depolarizations were required to initiate equivalent constrictions (fig. 5). Inspection of the membrane current records suggested that thresholds for activation of inward current had been elevated. These observations suggest that prazosin rather than blocking the stage of transmission involving the transmitter, antagonizes the mechanical responses to sympathetic nerve stimulation by a direct effect on arteriolar muscle excitability. The observations also suggest that it may be inappropriate to interpret action of a drug on such a complex series of events associated with neuromuscular transmission simply on the basis of the best catagorized pharmacological action of that drug.

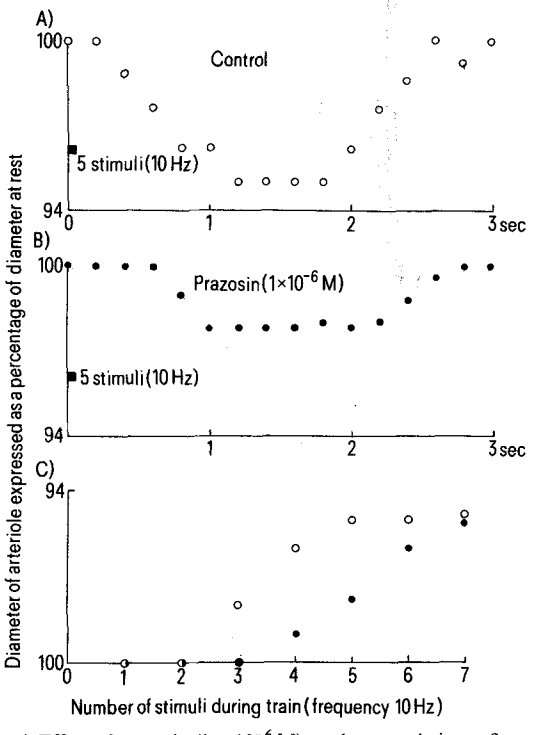

Figure 4. Effect of prazosin (1×10^{-6} M) on the constrictions of a guinea pig mucosal arteriole induced by trains of sympathetic nerve stimuli (train frequency 10 Hz). In A, a response in control solution is shown produced by 5 stimuli; *B* the same stimuli produce a smaller response in the presence of prazosin (1×10^{-6} M). The relationships between the peak amplitudes of arteriolar constriction in control (open circles) and in prazosin (1×10^{-6} M, closed circles) to an increasing number of sympathetic stimuli are shown in *C*. It can be seen that relationship is shifted to the left in prazosin but that the same maximal response can be achieved.

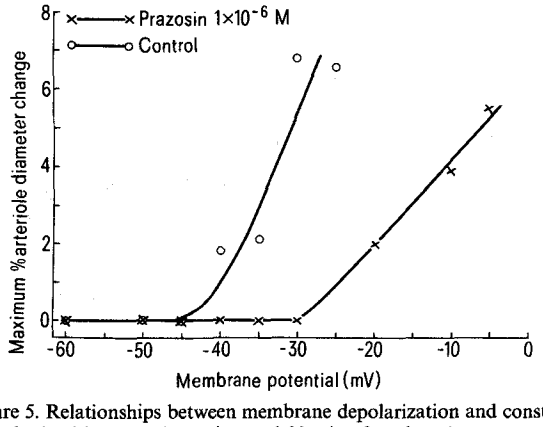

Figure 5. Relationships between membrane depolarization and constriction obtained in control solution and 30 min after changing to prazosin (1×10^{-6} M) containing solution. In each series the membrane potential of short segment of arteriole was 'jumped' from its resting value to a test value for 1 sec. The consequential change in diameter was measured from a video recording. Note that in prazosin larger membrane depolarizations are required to initiate similar constrictions. This would account for the changed number of sympathetic stimuli required to initiate equivalent constrictions to those obtained in control solution when in the presence of prazosin. That is more e.j.ps must sum to produce a larger depolarization to initiate an equivalent constriction.

Taken together then, these observations suggest that transmission in arterioles shares many similarities with transmission at other synapses. Whether it would be appropriate to extend this idea to other smooth muscles is not clear. A major difference between the junction potentials recorded from arterioles and visceral smooth muscle is the slow kinetics of transmitter-induced conductances in the latter. Since these appear to reflect the properties of the chemoreceptors activated, rather than diffusion[30], 'slowness' does not preclude receptor specialization. In caution however it is to be stressed that that idea of 'tight' synaptic control is usually associated with distinct morphological structuring between nerve terminals and effector cells. Such structuring has rarely been described for smooth muscle innervation patterns.

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Advances in the understanding of transmembrane ionic gradients and permeabilities in smooth muscle obtained by using ion-selective micro-electrodes

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Introduction

Classical techniques of ion analysis and radioisotope flux have indicated a basic similarity of the transmembrane ionic gradients in smooth muscle cells to those found in the much studied preparation at the other end of the cell size spectrum, the squid giant axon. Intracellular K^+ would appear to be high and intracellular Na^+ relatively low while intracellular Cl^- seemed to be considerably higher than that predicted from a passive distribution, that is from the membrane potential (E_m) and extracellular Cl^-[21,41]. However, theoretical determination of E_m from estimates of the intracellular concentrations and resting permeabilities using the constant field equation did not yield a value close to that measured by microelectrodes, but one some 30 mV too low[18]. This inequality led to the postulation of a significant contribution to E_m from electrogenic mechanisms, for example the Na^+-K^+ pump. However, it is clear that the accuracy of the theoretical determination is critically dependent upon the accuracy of the measurements of the ionic gradients and permeabilities. Brading[13] showed that, with a different interpretation of efflux data which yielded a notably lower estimate of both intracellular Na^+ and Na^+ permeability, E_m determined by the constant field equation could be close to that directly measured in both normal and hypertonic solution. Nevertheless, it would be unwise to use this agreement as grounds for the validity of these indirect determinations.

The development of intracellular ion-selective microelectrodes clearly provided the method for reliable measurements since not only is the intracellular *activity*