

Sympathetic neurotransmission: A new biological role for ATP?

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Neurotransmission from peripheral sympathetic nerves to smooth muscle is involved in the regulation of several important physiological processes. It has conventionally been held that noradrenaline (NA) is the only sympathetic neurotransmitter. Much recent evidence indicates, however, that not all sympathetic end effects may be mediated by NA alone. Instead, significant components may be mediated by adenosine 5'-triphosphate (ATP). In view of the far-reaching implications of this hypothesis, an examination is made in this paper of the pertinent evidence and avenues for future work are identified.

A review carried recently in this journal¹ has dealt with the electrical properties during neurotransmission of the smooth muscle end organs that are supplied by sympathetic nerves. At the same synapses, another question that is exciting much debate and interest is the identity of the neurotransmitter that mediates the responses of the smooth muscles, and this forms the subject of the present review. In this context there follows a brief description of the concept of chemical neurotransmission, with particular emphasis on sympathetic end organs.

Transmission of information at most contacts between nerve cells and their target effector cells, the process of neurotransmission, is chemical in nature^{2,3}. Neurons and their target cells are separated by a discrete gap, the synaptic cleft. Neurotransmission takes place by means of the release from the neuron of a chemical substance (the neurotransmitter) which then acts upon the postsynaptic cell^{2,3}. Since neurotransmission is predominantly chemical, very many physiological processes are susceptible to the influence of chemical agents which affect the release, action or metabolism of neurotransmitters. This principle in fact underlies a significant part of modern drug therapy^{4,5}. Clearly it is of fundamental importance to identify the neurotransmitters released at various synapses, in order to better understand physiological processes and their modulation by various factors.

At only a few kinds of synapses has the identity of the neurotransmitter been firmly established; at many others discovery or identification is still awaited^{2,3}. Amongst the latter are those involved in transmission from postganglionic sympathetic nerves to smooth muscle. This synapse is involved in the regulation of several impor-

tant physiological processes and parameters, including blood pressure, reproductive function, and airway regulation. Indeed, many smooth muscle-walled organs, such as arterioles and organs of the reproductive tract (including the vas deferens and seminal vesicle) receive a purely sympathetic innervation.

For 50 years now, postganglionic sympathetic nerves have been known to contain the neurotransmitter noradrenaline (NA)^{4,6}. The association between the anatomical origin and chemical properties of these nerves has historically been so entrenched that until very recently the terms 'sympathetic' and 'noradrenergic' were regarded as virtually synonymous. Consequently, our understanding of both the normal physiological control of these organs as well as the aetiology of malfunction has been prejudiced in terms of noradrenergic mechanisms^{4,5}.

A volume of recent evidence suggests, though, that at least some components of the responses of smooth muscle that result from sympathetic activation are produced not by the 'classical' neurotransmitter NA, but by a different substance released from the same innervation. There is now an impressive body of evidence that this second substance is the purine adenosine 5'-triphosphate (ATP). This departure in conception has important implications for the control of the biological processes in question⁷, and it is my aim in this review to set out the experimental evidence for it. To this end there is a detailed discussion of the case for a neurotransmitter function of ATP, in particular, the degree to which ATP fulfils the criteria for a neurotransmitter. Particular attention is paid to studies on the vas deferens and arterial vasculature, as it is in these end organs that investigations have been the most detailed and illuminating. There is also an assessment of the unresolved questions that will need to be addressed in future work. A final aim is to bring this area of inquiry to the notice of those who may be in a position to address these issues, and to invite their attention to problems that may be investigated fruitfully.

Non-noradrenergic sympathetic neurotransmission

To consider the evidence that some sympathetic end-effects are not mediated by NA, we take the example of the neurogenic (nerve stimulation evoked) responses of

the mammalian vas deferens, an organ of the male reproductive tract. The vas receives a purely sympathetic motor innervation from the vas deferens nerve (in turn supplied by the preganglionic hypogastric nerve). Nerve stimulation results electrically in transient depolarizations of membrane potential, the intracellularly recorded excitatory junction potentials (EJPs)^{1,8,9} which evoke action potentials, and mechanically it results in contraction of the smooth muscle mass¹⁰. The anatomically sympathetic innervation of the vas deferens bears, according to numerous physiological criteria, the hallmarks of a noradrenergic system. NA is present in the vas deferens of several species at a uniquely high concentration¹¹⁻¹³, and a dense noradrenergic innervation has been demonstrated histochemically¹⁴⁻¹⁶. In electron microscopic studies the axons and axon varicosities of the vas deferens nerve are found to include granular vesicles typical of NA-containing structures^{17,18}. The vas deferens contracts in response to externally applied NA¹⁹⁻²². Finally, superfusion with adrenergic neuron blockers such as bretylium and guanethidine, or chemical sympathectomy with the neurotoxin 6-hydroxydopamine, abolishes the responses to nerve stimulation, confirming that the motor transmitter indeed originates from the sympathetic nerves²³⁻²⁷. By analogy with other noradrenergic junctions, and on the basis of these physiological, pharmacological and histochemical properties, it was concluded by the pioneering investigators that NA was the only neurotransmitter released from the motor innervation of the vas deferens^{28,29}.

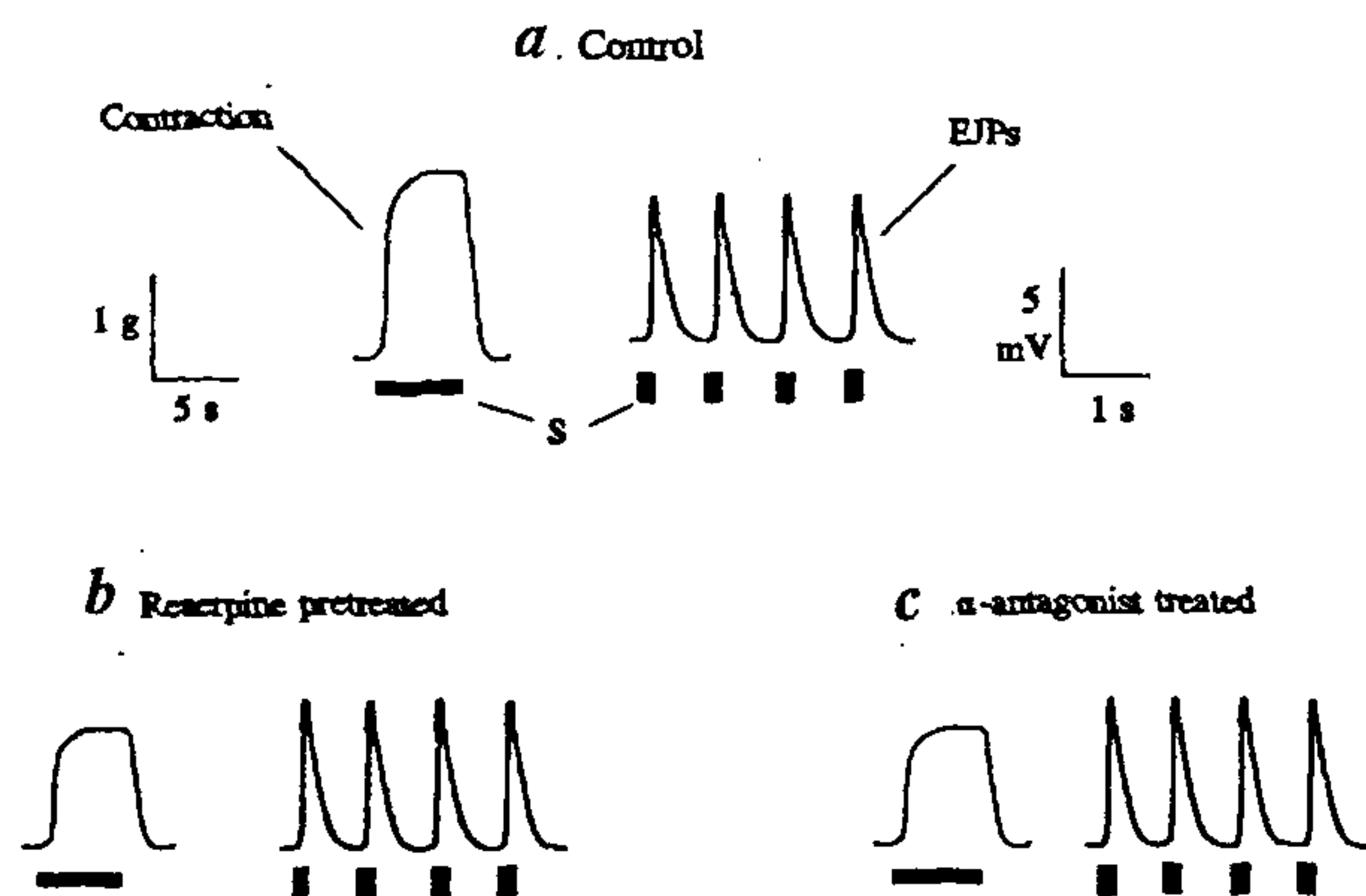


Figure 1. Schematic illustration of the effects of reserpine pretreatment and of α -adrenoceptor antagonists on the nerve stimulation-evoked contractile and electrical responses of the guinea-pig vas deferens. The contractions are elicited by short-duration trains of pulses (~5 s long, at frequencies of 10–20 Hz). EJPs are shown elicited by individual stimuli delivered at about 1 Hz. Both reserpine and α -antagonists reduce but do not abolish the contractions, and have no significant effect on the EJPs. Horizontal bars indicate period of repetitive stimulation eliciting contractions; vertical lines indicate individual stimuli that evoke EJPs. Calibration on right applies to EJPs and that on left to contractions. This figure, along with Figures 2 and 3, is based on the results in refs. 21, 22, 34.

Surprisingly, though, an equally large volume of evidence indicated that some components of neurotransmission in this organ might be produced by mechanisms unrelated to NA. The observations underlying this idea date back in fact to the earliest studies on the isolated vas deferens, when Hukovic¹⁰ found that the neurogenic contractions of the guinea pig vas deferens were reduced but never completely abolished by pre-treating the animals with reserpine, chemical which depletes almost completely the neuronal stores of NA^{30,31} (Figure 1 *b*). Similar observations on the contractions were made subsequently in several laboratories^{19,32-34}. The postjunctional electrical responses of the vas deferens, the excitatory junction potentials (EJPs), were also found to be resistant to the action of reserpine^{22,34-37} (Figure 1 *b*). Since it was known that reserpine at the doses in which it was used in these studies depleted NA by more than 99%, the responses that remained could not reasonably be attributed to NA.

In addition, the pharmacological profiles of the neurogenic contractions proved to be incompatible with purely noradrenergic mechanisms. Superfusion of the vas deferens with a variety of α - and β -adrenoceptor antagonists, which block the smooth muscle membrane receptors responsible for mediating the effects of NA⁴⁻⁶, failed to abolish completely the responses to nerve stimulation^{19,38-40} (Figure 1 *c*). Yet α_1 -adrenoceptor antagonists readily blocked the contractile responses to exogenous (externally applied) NA^{19,30,32,41,42}. The latter observation indicated that NA mediated a part of the contractile response through classical α -adrenoceptors (in this case shown to be of the α_1 subtype), and also that the α_1 antagonists were able to gain access to the receptor population that NA acted upon. But the component of contraction that was resistant to α -receptor blockade could arise from some other mechanism not involving NA. The EJPs, too, persisted in the presence of α -adrenoceptor blockers such as phenoxybenzamine or prazosin (Figure 1 *c*)^{22,28,43,44}.

To summarize, both reserpine pretreatment, which depletes neuronal stores of NA, and α -receptor blockade, which prevents NA from acting on the smooth muscle, failed to abolish either the contractions or the EJPs.

A clue to the cause of these discrepancies came from the observations on the neurogenic contractile response of the vas deferens when elicited by relatively prolonged stimulation pulse trains (e.g. 30 seconds duration at 8 Hz) rather than the conventional short ones (2–10 s duration)²⁵. The contraction then consisted of two distinct phases: a rapid phase (the 'twitch' contraction) and a slower phase (the 'phasic' contraction) (Figure 2 *a*). In his seminal report Swedin²⁵ reported that reserpine pretreatment abolished only the second slow phase of the response, leaving the first rapid phase intact (Figure 2 *b*). The rapid phase was also left intact, or even

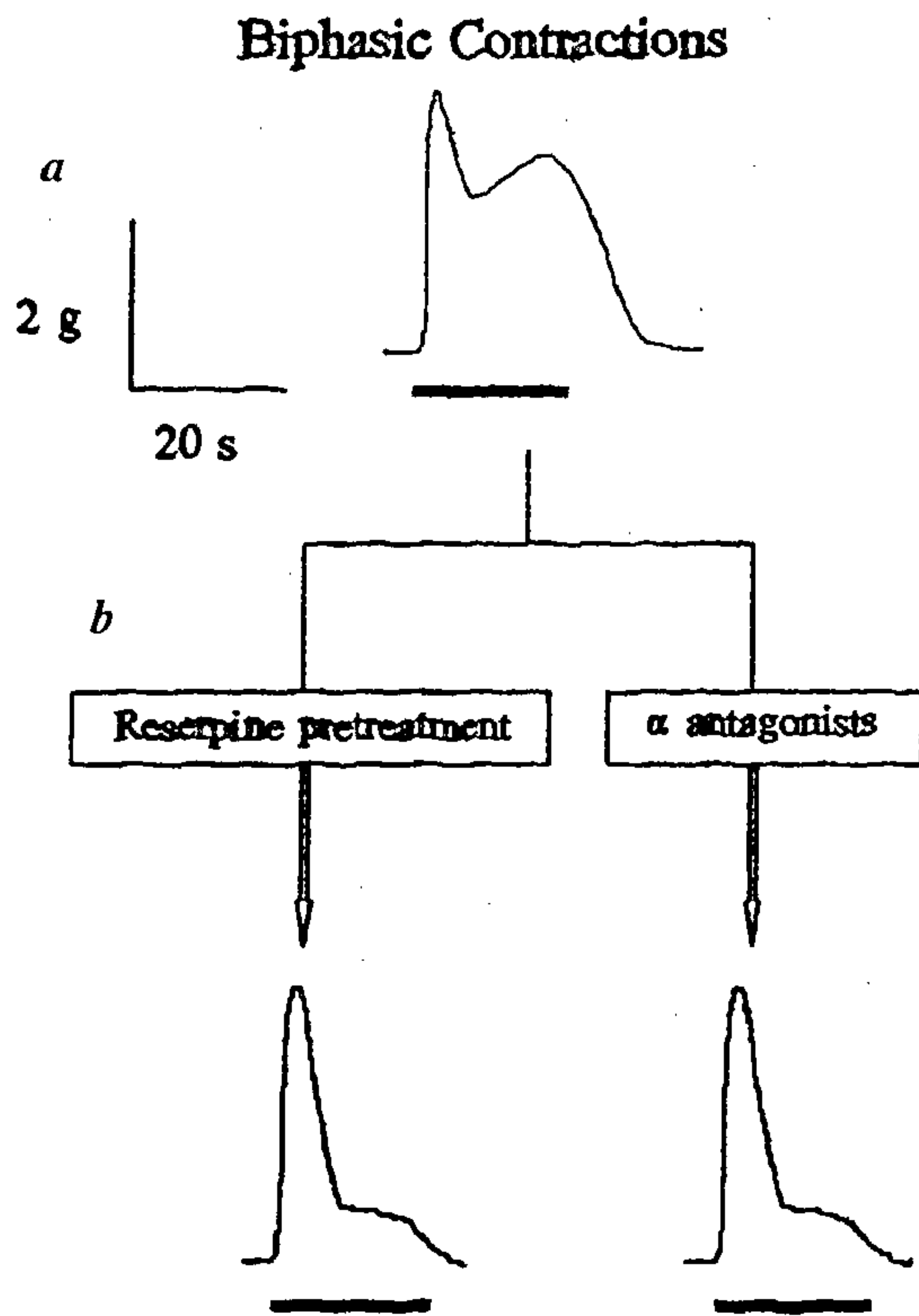


Figure 2. *a*, Biphasic neurogenic contractions of the vas deferens produced by long-duration trains of stimuli (20–30 s long, at frequencies of 10–20 Hz). Note the compressed time scale compared with that in Figure 1. *b*, Effects of reserpine pretreatment and of α -adrenoceptor antagonists on the biphasic contractions. Only the second, slow phase is affected.

enhanced, by the α -antagonist phentolamine, which by contrast powerfully antagonized the second slow phase (Figure 2*b*). Since the vas deferens is known to be innervated almost solely by sympathetic nerves, the possibility arose that a non-noradrenergic transmitter substance might be released along with NA from the same nerve fibres to mediate the rapid contractile phase. The susceptibility to reserpine pre-treatment and to α -adrenoceptor blockade of the second but not the first phase of the neurogenic contraction following trains of stimuli has been confirmed in a number of investigations^{21,22,26,34,44–46}.

On closer scrutiny, the contractile response of the vas deferens to just a single nerve stimulus was also discovered to have two phases⁴⁷. The components of the single-stimulus mechanical response behaved in the same manner as did those following repetitive stimulation, i.e. the second phase was sensitive to both reserpine pre-treatment and to α -adrenoceptor antagonists, whereas the first phase was not^{45,47}. The most parsimonious ex-

planation of these phenomena is that although NA may indeed be the neurotransmitter of the second, slow phase of the neurogenic contraction of the vas deferens, the first, rapid phase of the contraction, and the EJPs of the vas deferens, may be produced by non-noradrenergic mechanisms, that is, by a second, non-noradrenergic 'co-transmitter'²² released from the sympathetic nerves. (This is a revolutionary biological concept: ever since the discovery of neurotransmission it had been believed that an individual nerve cell releases just one transmitter.) The question arises: what substance might the co-transmitter be?

Purinergetic transmission

Following a series of experiments in different laboratories in which none of the 'classical' transmitters including acetylcholine, 5-hydroxytryptamine, histamine and dopamine, were found to possess transmitter-like activity at the sympathetic neuroeffector junction, the most likely candidate to emerge in the role of a sympathetic 'co-transmitter' is the purine, ATP. It seems at first sight a matter of surprise that ATP, so well known for its intracellular energy-related functions⁴⁸ should be put to use as a neurotransmitter. Nevertheless the idea that ATP might also act as a neurotransmitter is not new; it is about 40 years old, among the first such suggestions, having appeared in the 1950s^{49,50}. Burnstock and his colleagues⁵¹ were the first to attribute to ATP a neurotransmitter function in the autonomic nervous system. But the suggestion that it is involved in sympathetic neurotransmission is a relatively recent one, going back to the early 1980s. The neurotransmitter role of ATP in a number of tissues has been considered and widely discussed elsewhere^{7,52–55}, and recently it has been summarized for the central nervous system⁵⁶. These reviews may be consulted for certain details of purinergetic transmission that will not be elaborated here, such as the structures of purinergetic receptors and their transduction mechanisms.

ATP as a sympathetic neurotransmitter

For a putative substance to be considered unequivocally the transmitter at a neuromuscular junction, several criteria need to be satisfied, of which the following are of greatest importance^{2,57}.

- i) The substance must be shown to be stored in the pre-junctional nerve fibres, and released from them following nerve stimulation.
- ii) Upon external application to the muscle it should produce responses, both electrical as well as contractile, similar to those following nerve stimulation. In the case of the vas deferens, therefore, externally applied ATP

should mimic the EJP as well as the rapid phase of the neurogenic contraction.

iii) Both the neurogenic responses and those evoked by the substance applied externally should be blocked by the same antagonists.

iv) A change in external conditions, such as the temperature or pH, should produce matching alterations in neurogenic responses and those elicited by the substance applied externally.

v) A demonstrable mechanism of inactivation for the putative neurotransmitter should exist at the synapse being studied; and interference with inactivation should lead to potentiation and prolongation of the postjunctional responses.

We may now see against the above criteria the pertinent experimental observations that help us to evaluate the degree to which ATP satisfies its putative function as a sympathetic co-transmitter.

Storage and release

The storage of NA and ATP in sympathetic nerve axons, and indeed in the same intra-axonal vesicles, was widely documented in biochemical studies^{18,31}. ATP was shown to be co-stored with NA in these vesicles. To establish that ATP is released from these vesicles by nerve stimulation is not straightforward. Experiments are usually performed on isolated nerve-muscle preparations, and whether the ATP detected after stimulation originates from the nerve fibres themselves or from extraneuronal tissue (e.g. consequent to the action of neurotransmitter), needs to be resolved. The initial suggestion that ATP is released from the sympathetic innervation of the vas deferens is due to Westfall *et al.*⁵⁸. They demonstrated the release, following nerve stimulation, of tritium from vasa deferentia preincubated with ³[H]-adenosine, suggesting the release of ATP from the sympathetic nerves (assuming the incorporation of the label into intra-axonal ATP). But in these studies the possibility of extraneuronal release of ATP could not be dismissed. More convincingly, Lew and White⁵⁹ monitored the release of endogenous ATP by detecting the luminescence produced by its reaction with firefly luciferin-luciferase included in the incubation medium. They showed that the tetrodotoxin-sensitive (and hence nerve action potential produced) postjunctional contractile responses were accompanied by neurogenic release of ATP; however when contractions were produced by exogenously applied phenylephrine (an α_1 noradrenergic agonist), ATP was not released concomitantly. Thus the ATP that is released consequent to nerve stimulation seems to originate from the sympathetic innervation and not from the postjunctional smooth muscle cells, for instance following the activation of α_1 receptors by NA. Other laboratories have also

documented the neuronal release of ATP from the sympathetic innervation following activation⁶⁰⁻⁶³.

Cultured sympathetic neurons offer a preparation free from the uncertainties of whole tissue, because no post-receptor or other extraneuronal elements are present to complicate interpretation. Recently von K ugelgen *et al.*⁶⁴ have demonstrated the co-release of NA and ATP from cultured chick sympathetic neurons, lending robust support to the hypothesis that the putative co-transmitters are stored in and released from the same sympathetic nerve fibres. It has now become possible to make indirect measurements of the release of NA following individual stimulus delivered to the nerve, using the electrochemically determined NA oxidation current at thin carbon fibre electrodes placed near neuronal varicosities⁶⁵⁻⁶⁷. Should a method with similar resolution be developed for measurement of pre-pulse ATP release, it would greatly enhance our understanding of the release kinetics of these transmitters, and of the issue of whether or not they are truly 'co-released' at the level of the individual varicosity when it is invaded by a single nerve impulse.

Antagonist sensitivity

Receptor antagonists have played a significant role in the delineation of neurotransmission processes^{4,5}, as outlined above for the evolution of ideas against purely noradrenergic transmission from sympathetic nerves. For noradrenergic receptors, antagonists have been known since the early part of this century. But for the postjunctional excitatory purinergic receptors in the vas deferens and blood vessels (the P_{2x} receptors^{68,69}) no specific blockers were available until the early eighties, which saw the introduction of two such agents. The first was the light-activated, irreversibly binding ATP analogue arylazido aminopropionyl ATP (ANAPP₃) which in 1980 was shown by Hogaboom *et al.*⁷⁰ to possess the properties of a specific P_2 -purinoreceptor antagonist. Subsequently, Kasakov and Burnstock⁷¹ reported that another ATP analogue, α,β -methylene ATP (α,β -meATP), produced a slow desensitizing action on P_2 purinoreceptors and could be used thereby to antagonize the actions of ATP. Using these substances it was shown that:

(i) The seemingly non-noradrenergic neurogenic responses of the vas deferens, i.e. the rapid phase of contraction and the EJPs, were specifically abolished by either the blockade (by ANAPP₃) or the desensitization (by α,β -meATP) of the postjunctional P_2 purinoreceptors^{22,59,72} (Figure 3 a, b).

(ii) The contractile as well as electrical responses to exogenously applied ATP were similarly abolished by both agents. However the P_2 receptor blockers left unaffected by NA-mediated slow phase of contraction^{21,22,44,72} (Figure 3 a, b).

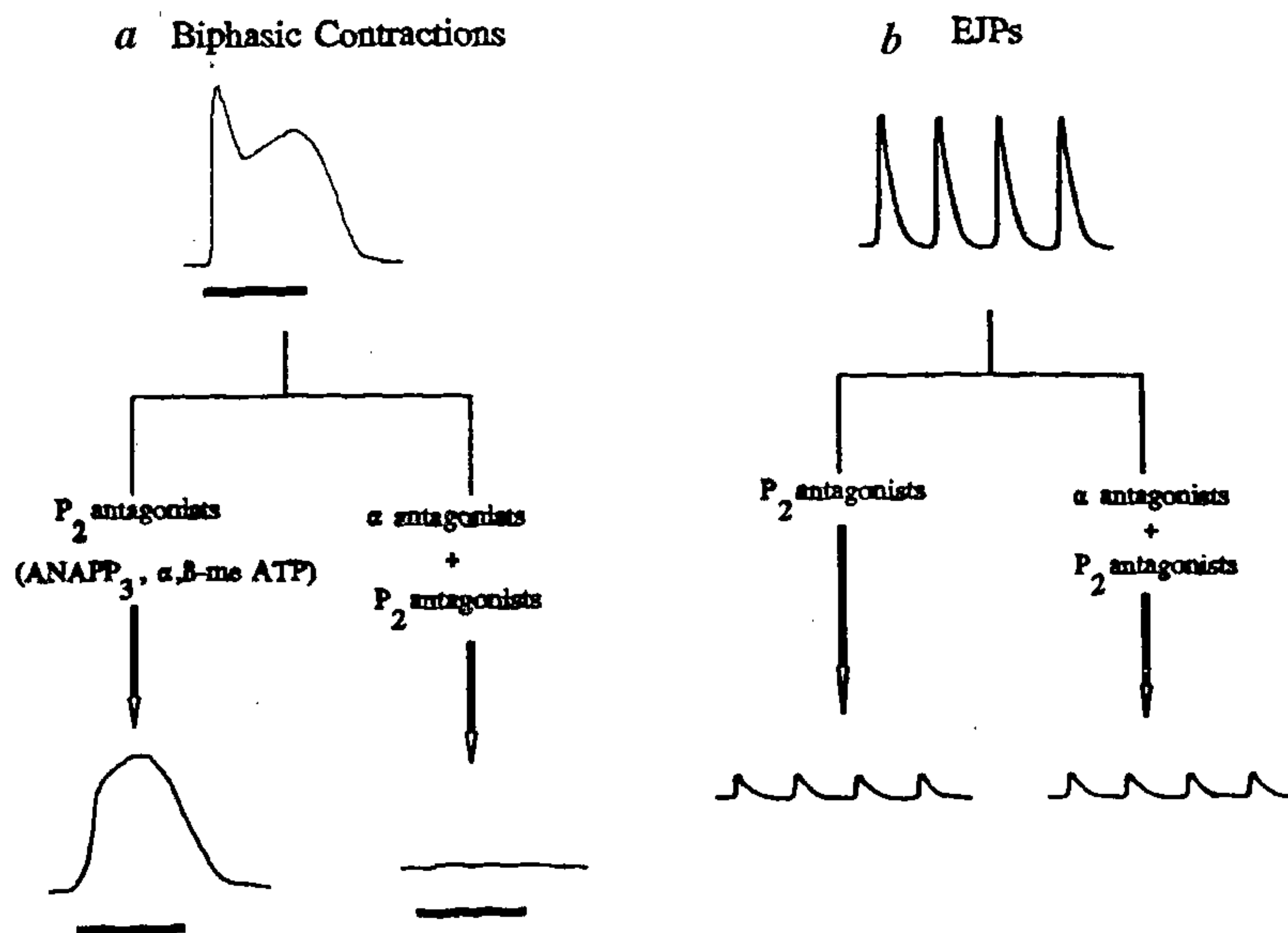


Figure 3. Depiction of the effect of P₂-purinoceptor antagonists alone and of a combination of α-adrenoceptor and P₂-purinoceptor antagonists on the biphasic contractions (a) and the EJPs (b) of the vas deferens. Blockade of purinergic transmission affects only the first, rapid phase of contraction while blockade of both purinergic and adrenergic receptors affects both phases. Purinergic receptor blockade alone is sufficient to suppress EJPs.

(iii) Significantly, when either ANAPP₃ or α,β-meATP was used together with an α-antagonist, e.g. prazosin, contractions were in most cases fully abolished (Figure 3a). This indicated that transmission by NA and ATP together was sufficient to account for all sympathetically evoked post-junctional responses (but see ref. 73 for suggestion of a third transmitter in the vas deferens of young guinea-pigs).

The experimental use of ANAPP₃ or of α, β me-ATP is problematic, that of the former procedurally since it is photoactivated and irreversible, and that of the latter because the antagonism is elicited through desensitization, which may alter receptor properties in subtle ways. In recent years several other substances have been reported to exhibit more or less selective, reversible blockade of P_{2x} purinoceptors. They include trypan blue⁷², pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS)^{75,77} and the erstwhile trypanocidal agent suramin⁷⁷⁻⁸⁰. It is hoped that substances such as these will facilitate detailed characterization of purinergic neurotransmission by acting as starting points for the development of ever more specific P_{2x} purinoceptor antagonists. Of these, suramin seems to be the most promising. Its antagonist activity was first reported in 1988 (ref. 77), and since then its use in blocking purinergic responses has proliferated⁸¹⁻⁸⁵. The use of suramin in sympathetic end organs such as the vas deferens and blood vessels has confirmed the ideas obtained earlier with ANAPP₃ and α,β-meATP, and has put on surer footing the neurotransmitter role of ATP⁷⁷⁻⁸⁵.

Actions of exogenous ATP

When ATP is applied externally to the vas deferens it produces contractions that are similar to the first rapid phase of neurogenic contraction, the phase which is resistant to adrenoceptor but sensitive to purinoceptor blockade. Exogenous NA also evokes a contraction, but one that is comparatively sluggish and resembles the slower α₁-antagonist sensitive phase of the neurogenic contraction. The responses to ATP and to NA have the expected pharmacological sensitivities, with the ATP contractions being blocked selectively by purinoceptor antagonists and the NA contractions by α₁ blockers²².

Contractile responses of a muscle are the end effect of a series of processes beginning with neuromuscular transmission and including muscle action potential generation and propagation, excitation-contraction coupling, and activation of the contractile machinery. The very first event to occur during excitatory neurotransmission is the generation by the neurotransmitter of depolarizations such as the EJP. Therefore mimicry of the neurogenic electrical event, the EJP, provides a more stringent test of the neurotransmitter function of a proposed substance.

The electrical effects of local application of ATP through microelectrodes were first investigated on the vas deferens in the early eighties^{22,44}. It was shown that ATP produced depolarizations (ATP potentials) that were similar in shape to EJPs in the guinea pig vas deferens, but they did not examine closely the accuracy of

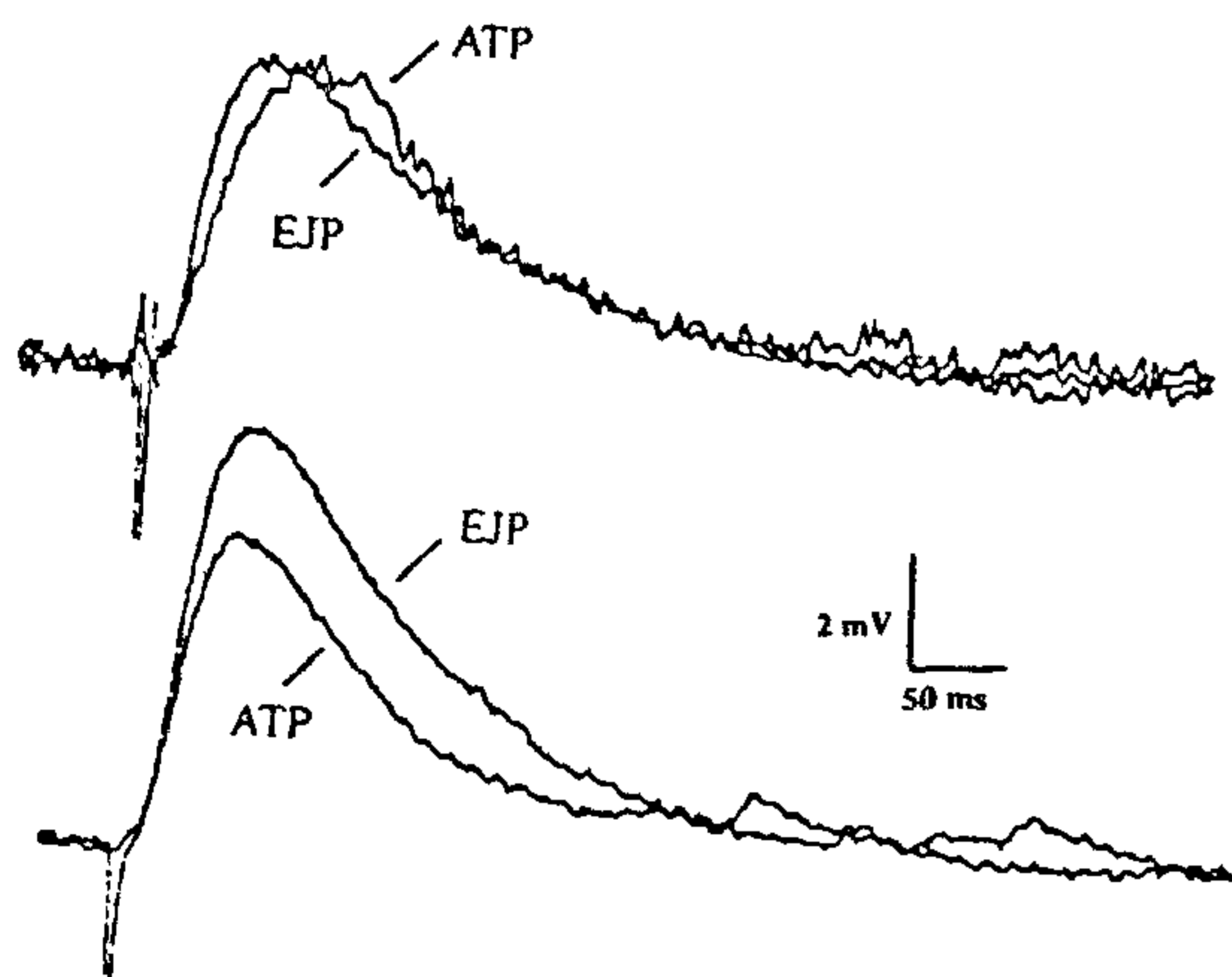


Figure 4. Comparison of neurogenic EJPs with ATP potentials produced by ATP (10^{-4} M) applied externally from a micropipette positioned within about $100\ \mu\text{m}$ of the recording microelectrode. Note the close correspondence in amplitudes and time courses. The sharp downward vertical deflections are the stimulus artifacts preceding the EJPs, and the small brief depolarizations are spontaneous excitatory junction potentials (SEJPs). Duration of ATP application was 6 min. Each pair of records are taken from a different tissue.

replication. Subsequent experiments showed⁸⁶ that the application of brief pulses of ATP, lasting less than 10 min, onto the surgically cleaned surface of the vas deferens produced ATP potentials that were virtually identical in all respects to EJPs evoked simultaneously in the same tissue. Examples of this correspondence are shown in Figure 4. It is evident that EJPs and ATP potentials have very similar rise times, durations, time constants of decay, and amplitudes. The striking similarity indicates that the mechanism of action and of inactivation of the endogenous neurotransmitter must be very similar to that of the externally applied ATP.

In contrast to the effect of exogenous ATP, exogenous NA applied focally did not produce postjunctional depolarizations. This, together with the accurate replication of the EJP by ATP, renders it likely that ATP, and not NA, is the sympathetic transmitter producing the EJP^{22,86,87}. Strengthening this conclusion, it has been shown using extracellular recording that the transmembrane currents in smooth muscle that underly the EJP, the excitatory junction currents (EJCs), are also very similar when they are produced either by locally applied ATP or as a result of nerve stimulation⁸⁸.

Effects of external conditions

The effects of temperature on sympathetic neurotransmission have received considerable attention. Both the neurogenic contractile response^{19,47,89,90} as well as the EJPs^{91,92} of the vas deferens are prolonged when temperature is lowered from 35 to 25°C. It was therefore of

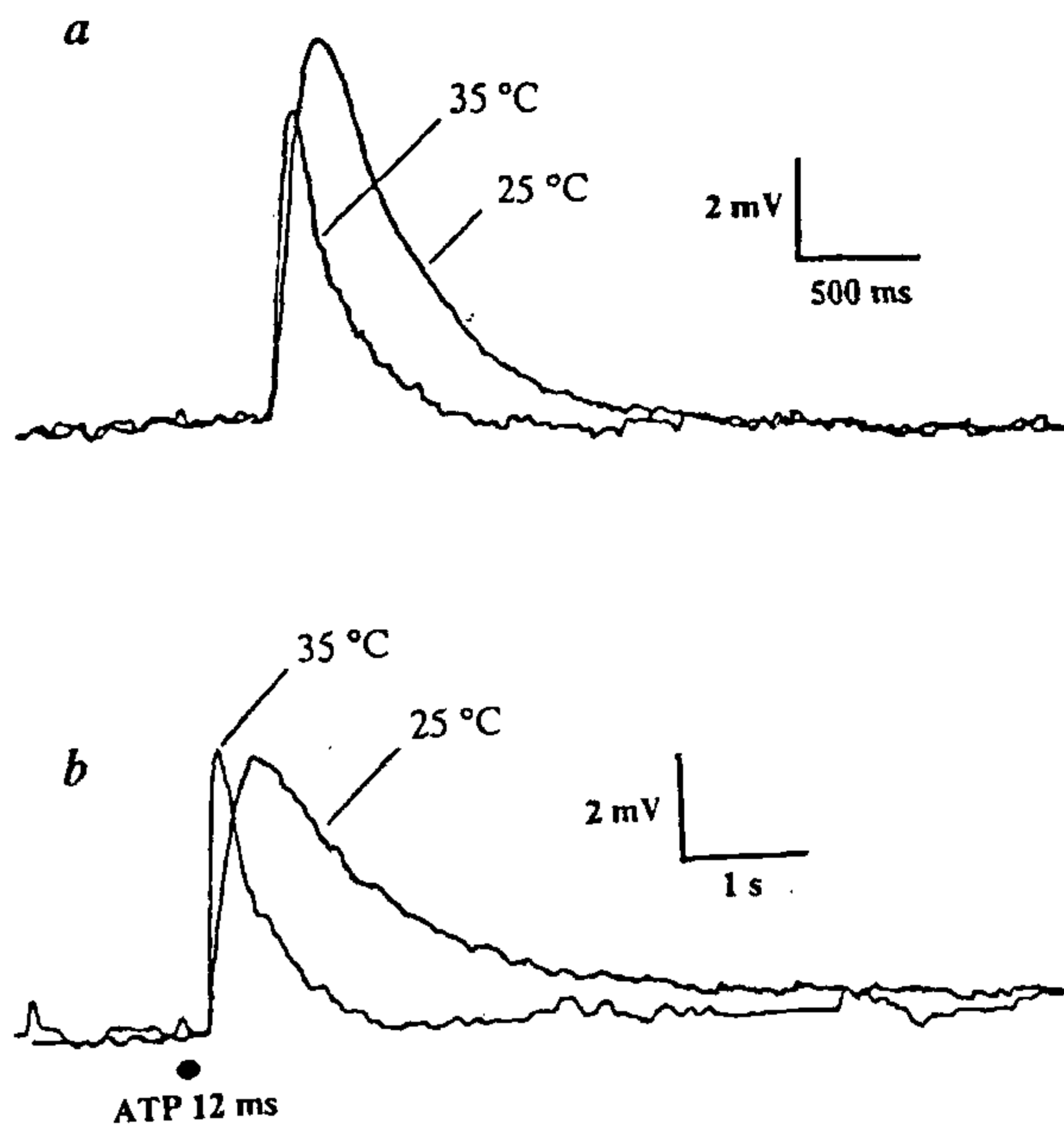


Figure 5. Effect of temperature on EJPs (a) and ATP potentials (b). EJPs at 25°C are not only prolonged but also potentiated, the mechanism for which is not known. Duration of application of ATP is as indicated.

interest to see whether responses evoked by exogenous ATP would be affected likewise. The comparative effects of temperature on EJPs^{86,93} and on ATP-evoked depolarizations⁸⁶ have been investigated. It was found, as shown in Figure 5, that both EJPs and ATP potentials were prolonged in a similar manner by cooling. The rise time as well as the decay time constants of the depolarizations were lengthened, and the Q_{10} for the temperature sensitive decays of both EJPs and ATP potentials was similar. It remains to be seen whether changes in other external conditions, e.g. pH and the ionic composition of the extracellular fluid, have parallel effects on both events.

Inactivation

The mechanism of inactivation of ATP following its neural release is yet to be fully elucidated. ATP is known to be degraded enzymatically both within cells and extracellularly by different kinds of ATPases^{94,95}, and it is reasonable to propose that nerve released ATP is removed by enzymatic hydrolysis. This possibility could be tested directly if the enzyme responsible for the synaptic inactivation of ATP were known and characterized, and if inhibitors for it were available. But, there is as yet no definitive information on these issues. An indirect test of the possibility can however be carried out by

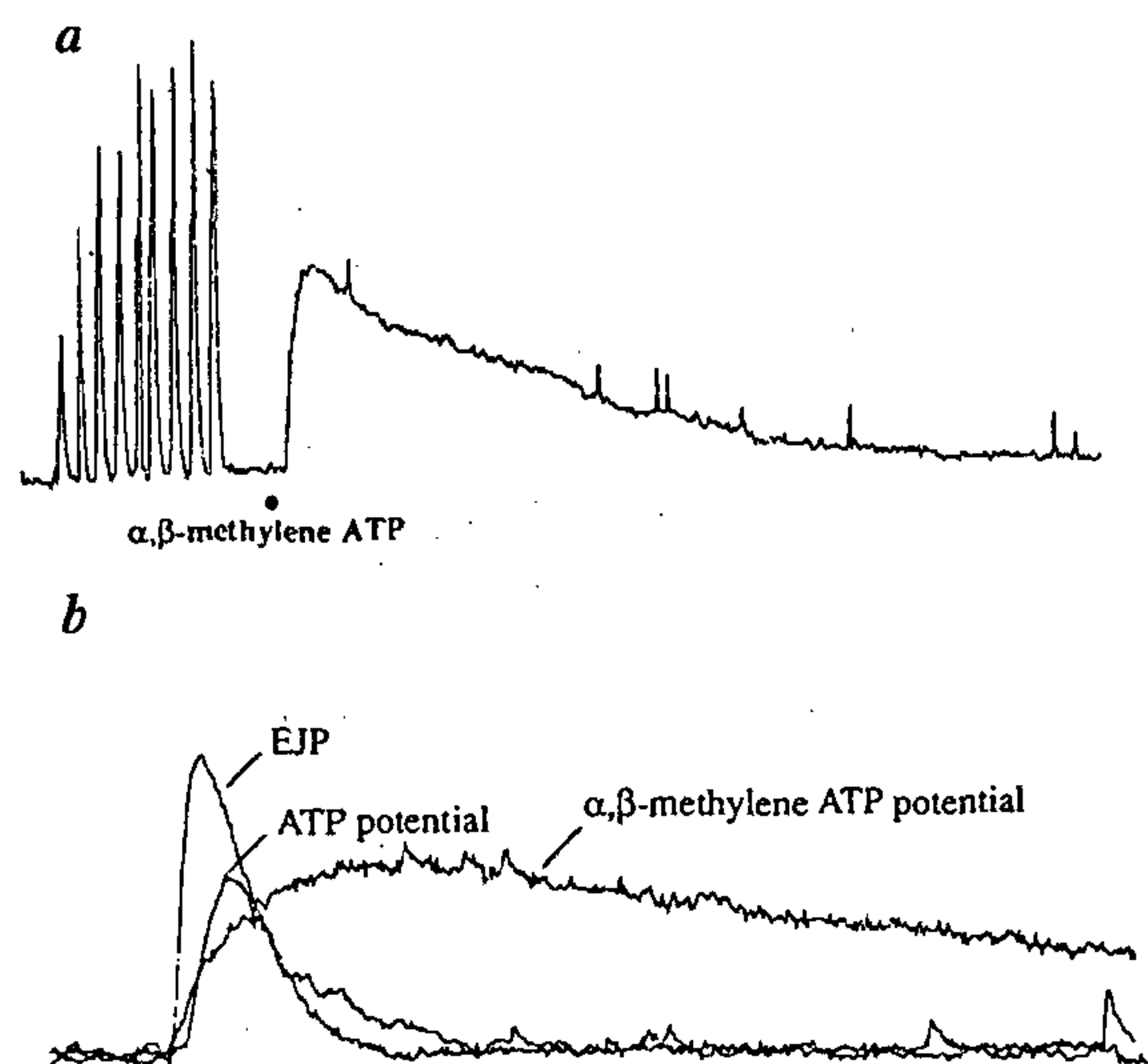


Figure 6. Relative time courses of EJPs, ATP potentials and α,β -meATP potentials. *a*, A train of EJPs followed by a depolarization produced by focal application of 10^{-6} M α,β -meATP. The EJPs show facilitation in amplitude as is normally the case for the first few EJPs in a train. The small brief depolarizations are SEJPs. *b*, Showing the very slow rising phase of an α,β -meATP potential compared with the time courses of an EJP and an ATP potential. ATP and α,β -meATP potentials were obtained using pulsed applications of durations between 6 and 10 min. Calibrations: Voltage: 2 mV for *a*, 4 mV for *b*, Time: 5 s for *a*, 0.5 s for *b*.

using a nondegradable analogue of ATP which activates the P_{2x} purinoreceptors but escapes enzymatic destruction. An example is the desensitizing blocker α,β -meATP (since it activates the receptors before desensitization sets in). This substance produces contractions³⁴ and depolarizations⁸⁶ that are 10–30 times more prolonged than the analogous events evoked by ATP. Examples of the prolonged responses are shown in Figure 6. One may reasonably conclude that the longevity of responses to α,β -meATP owes to the relatively prolonged presence of this agent in the extracellular space by virtue of its resistance to hydrolysis. The electrical responses to α,β -meATP were shown not to be artefacts, arising for instance from variations in placement of the microelectrode, since when both ATP and α,β -meATP were applied from the same microelectrode, biphasic depolarizations were obtained, an initial rapid phase mediated by ATP and a slower second phase due to α,β -meATP (Figure 7 *a*)⁹⁶.

Observations of this nature are consistent with the idea of enzymatic breakdown of ATP, perhaps by an extracellular ATPase. The evidence parallels that obtained at the skeletal neuromuscular junction, where the neurotransmitter acetylcholine (ACh) acts upon post-junctional nicotinic receptors and is inactivated by the

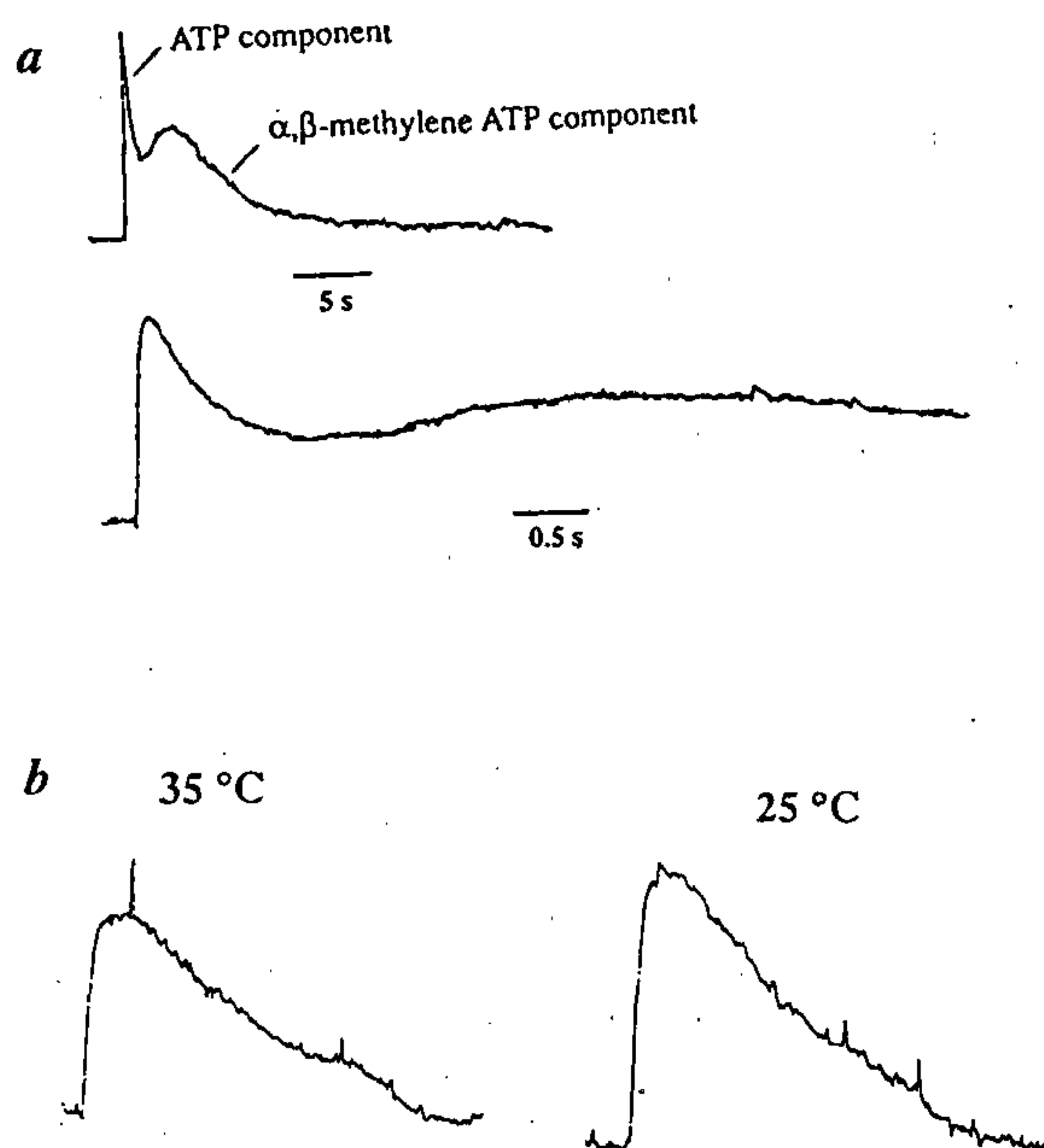


Figure 7. *a*, Membrane potential change produced by a mixture of 10^{-4} M ATP and 10^{-6} M α,β -meATP applied from the same micropipette, showed on a compressed time scale (upper panel) and an expanded one (lower panel). *b*, α,β -meATP potentials at two temperatures, showing the lack of effect of temperature on the durations of these potentials. Calibration same as in upper panel of *a*.

junctional enzyme acetylcholinesterase (AChE). Here, too, substances such as carbachol, which act upon the nicotinic receptors, as does ACh, but are resistant to destruction by AChE produce depolarizations that are considerably potentiated and prolonged compared with those produced by ACh⁹⁷.

In skeletal muscle, investigation of cholinergic transmission was greatly aided by the availability of specific inhibitors of AChE, such as neostigmine^{98–100}. Specific inhibitors of the junctional ATPase presumably involved in the degradation of extraneuronal ATP are not yet available. However, since enzyme action is temperature sensitive, the proposed enzymatic hydrolysis of ATP at sympathetic junctions may also be suggested to be a temperature-sensitive process. Indeed, ATPases in general are known to be temperature sensitive¹⁰¹. As mentioned above, EJPs and ATP responses are prolonged by cooling. It is possible, then, that the prolongation at low temperature of EJPs and ATP potentials might arise from inhibition of the ATPase resulting from cooling⁸⁶.

One can test this hypothesis further by the use of α,β -meATP and the following argument. In the case of an

activator which is not affected by transmitter removal mechanism, the responses that it produces should be insensitive to inhibition of the removal mechanism. Such has been found to be true at other synapses. Thus in skeletal muscle, the responses evoked by the AChE-resistant agents, carbachol and decamethonium, do not change when AChE is inhibited⁹⁷. By analogy, if temperature affects junctional ATPase, one would expect that the responses to α,β -meATP should not be temperature sensitive even though the responses to ATP are. Experimental observations support this prediction⁸⁶: although lowered temperature prolonged EJPs and ATP potentials, it did not significantly affect the duration of the depolarizations produced by α,β -meATP (Figure 7b).

These observations strongly suggest, but do not establish, the idea that enzymatic hydrolysis of ATP may be the principal mechanism limiting its junctional lifetime after release. Support for this notion has come from recent experiments¹⁰² in which the membrane currents elicited by ATP and its analogues in enzymatically isolated single smooth muscle cells were examined. Dispersed cells are thought not to have extracellular ATPase associated with their external surfaces, since the overlying extracellular matrix has been removed. Under these conditions it was found that ATP and α,β -meATP produced membrane current responses at equal potency and of similar time course. Thus in the absence of the putative enzymatic removal mechanism, ATP produces responses that emulate those of its nondegradable analogue, much the same as ACh at skeletal muscle end plates produces responses similar to those of carbachol once AChE has been inhibited^{97,98}. Validation of this hypothesis will have to await the discovery of specific extracellular ATPase inhibitors. The hunt is now on for such a substance^{95,103}, and in the next few years it will be of great interest to see if one is found, and if it can be shown to exert the anticipated effects. This will place the purinergic transmitter hypothesis on rather firmer footing.

Vascular and other tissues

Evidence for a neurotransmitter function for ATP in sympathetically innervated vascular tissue will now be summarized. It is along similar lines to that obtained in the vas deferens. Accordingly, the electrical and mechanical responses of many blood vessels to sympathetic nerve stimulation are not completely abolished by reserpine pre-treatment or by α -adrenoreceptor blockers. The resistant components of the responses are, however, generally abolished after exposure to either α,β -meATP or ANAPP₃, and can be mimicked by exogenous ATP^{87,104-112}. Owing to these and other lines of evidence, purinergic as well as noradrenergic components

of sympathetic neurotransmission seem to exist in the vasculature.

Apart from its role in sympathetic function in the vas and the vasculature, evidence is also fast accumulating for transmitter-like actions of ATP in other autonomic end organs⁷. These include the cat colon⁸³, chicken rectum¹¹³, the bladder of several species^{114,115}, and guinea-pig stomach¹¹⁶ and colon¹¹⁷.

It is interesting to note that ATP may be a transmitter at synapses in the central nervous system^{56,84,118-121}. Edwards *et al.*¹¹⁹ and Evans *et al.*¹²⁰ have presented convincing evidence that ATP mediates the fast synaptic currents associated with neurotransmission in rat medial habenula and guinea pig cultured coeliac ganglion neurones, respectively. Zimmerman⁵⁶ has summarized these developments. This new area of interrogation opens up new and exciting possibilities, as ATP could be involved in the central regulation of physiological events in addition to transmitting, in the periphery, the centrally issued commands.

Problems with the purinergic hypothesis, and open questions

Although the evidence for purinergic transmission is gaining in strength, it has not found unquestioning acceptance. Indeed, the results pointing to non-noradrenergic mechanisms (detailed in the section '*Non-noradrenergic sympathetic neurotransmission*' above) have been sought to be reconciled within the framework of noradrenergic transmission. Some have gone so far as to invoke the existence of a unique, hitherto unknown, kind of adrenergic receptor, the γ -receptor^{122,123}, a suggestion that has fuelled considerable debate¹²⁴⁻¹²⁷. The demerits of the γ -receptor hypothesis are that the receptor itself is purely a conjectural construct, and it is untestable because no antagonist is known that will block it. In comparison the purinergic hypothesis offers to date the most economical explanation of the range of experimental observations before us, rendering any alternative hypothesis perhaps unnecessary³⁷.

There are other problems with the hypothesis of cotransmission by NA and ATP. For instance if ATP mediates the electrical events, it remains to be demonstrated, as has convincingly been done for ACh at the skeletal neuromuscular junction¹²⁸, and that the amount of ATP stored in a vesicle is sufficient to account for the effect of quantal release, i.e. a spontaneous EJP. At present the best estimate for the ATP content of a vesicle is quite low, falling between 20 and 60 molecules¹²⁹. It is not clear whether this is sufficient to account for the generation of the SEJP. But since the determination of ATP content is itself subject to considerable uncertainty, ATP being a very labile molecule, it is possible that new improved estimates will afford a better match.

The details of co-storage of ATP and NA, such as energy requirements and vesicle membrane pumping mechanisms^{18,56,130}, need to be elucidated. Concerning postjunctional responses, it remains to be shown that externally applied ATP can mimic the SEJP of smooth muscle in addition to the EJP, although there is some indirect evidence for this⁸⁸. The development of selective purinoreceptor antagonists, and the differentiation of the effects of ATP from those of its analogues and breakdown products at the receptors, would help isolate the actions of ATP as against those of other agonists. The identification of the ecto-ATPases which subserve the removal of ATP during neurotransmission will greatly aid the clarification of purinergic junctional mechanisms. So will the development of specific inhibitors for these enzymes. Substances such as Evans blue seem to hold considerable promise in this respect¹³¹. Finally at the microelectrophysiological level it needs to be shown that the ion channel activation events produced by ATP at the P_{2x} purinoreceptors are of a nature that can fully account for its macroscopic quantal actions, i.e. the SEJCs and SEJPs. The techniques of recording (patch clamp) or inferring (fluctuation analysis) single channel transitions produced by ATP should help answer these questions.

Concluding remarks

50 years after the discovery of NA we cannot confidently equate, as Brown (ref. 132) could, NA with

'the' sympathetic neurotransmitter. Many end organ sympathetic responses are now proving to be more amenable to explanation by purinergic rather than noradrenergic mechanisms. It seems inescapable now to invoke co-transmission by NA and ATP to explain the range of observations before us. The idea of co-transmission itself, although first put forth only about 20 years ago¹³³, is now firmly in place for a wide variety of autonomic junctions¹³⁴. Specifically for sympathetic nerves, we may now consider a depiction of current knowledge such as the one in Figure 8, in which ATP and NA are both released from the same varicosity and act upon the smooth muscle cells via different pathways to exert their effects. The effects in this scheme are shown to be independent; but there is the possibility, yet to be fully explored, that each transmitter may also subtly modulate the actions of the other. Another complication not illustrated is that yet another co-transmitter may also be involved.

In summary, although at selected junctions and for certain postjunctional responses the evidence for ATP being a neurotransmitter appears compelling, further work needs to be done before the idea can be accepted unequivocally. And if co-transmission by NA and ATP proves to be a governing principle of sympathetic neurotransmission, it will necessitate a fundamental change in our conception of sympathetic and noradrenergic function, both in the central and the peripheral nervous systems.

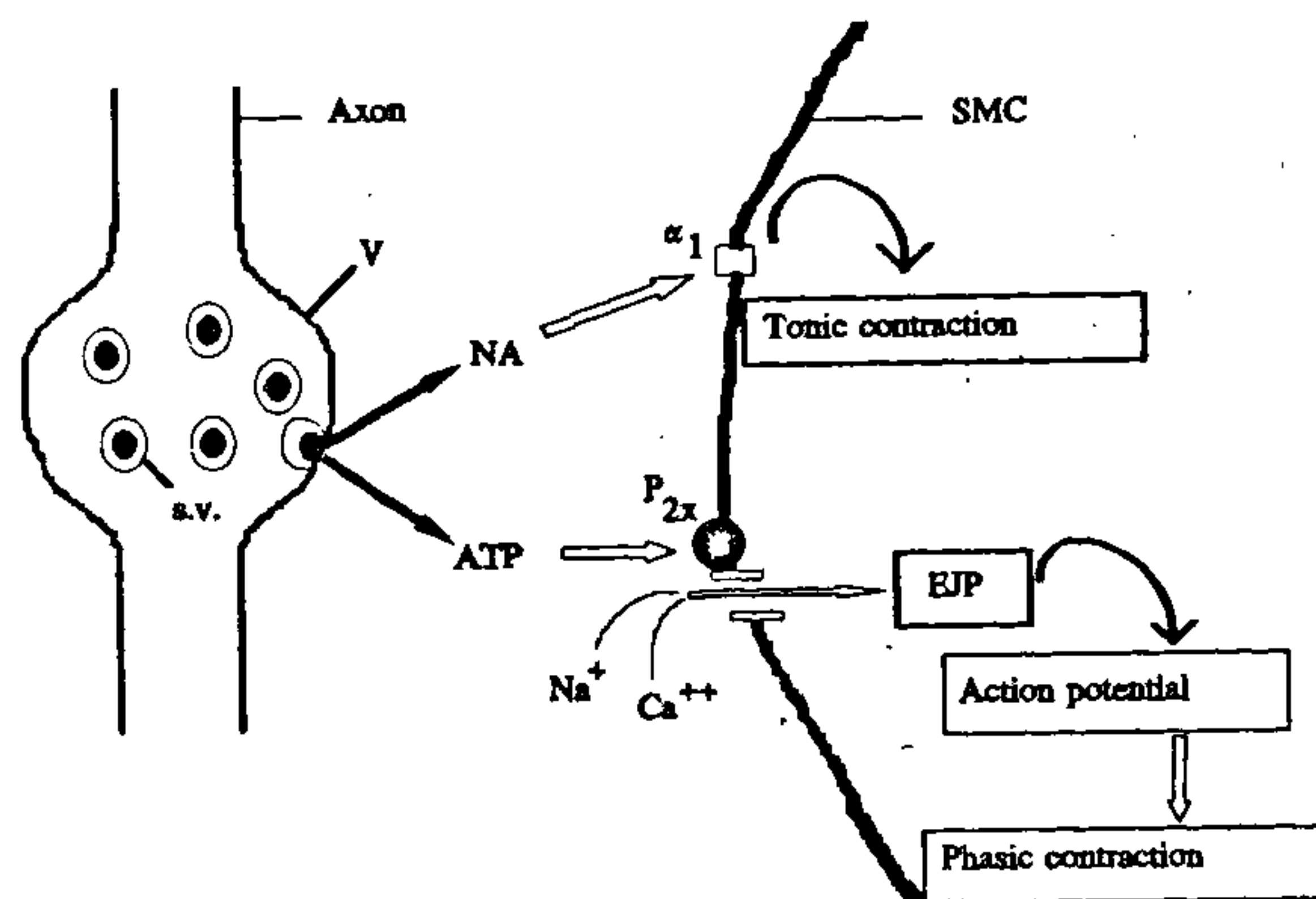


Figure 8. Sympathetic excitatory co-transmission by NA and ATP in the vas deferens: salient features. ATP and NA are co-stored in synaptic vesicles (s.v.) clustered in a sympathetic terminal axonal varicosity (V). Both substances are exocytosed into the synaptic cleft when transmitter release is induced (shaded arrows). NA acts upon postjunctional α_1 adrenoreceptors located in the smooth muscle cell (SMC) membrane, while ATP acts on P_{2x} purinoreceptors. Through these receptors which are thought to be linked to, or themselves constitute, ion channels permeable to Na⁺ and Ca⁺⁺, ATP generates EJPs. EJPs can give rise to action potentials which in turn are thought to set off the rapid phase of contraction. By a mechanism that probably involves Ca⁺⁺ mobilization, noradrenergic activation produces the slow phase of contraction. The fast and slow contractions produced by the two co-transmitters sum to produce the kind of biphasic contractions shown in Figures 2 and 3.

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RESEARCH ARTICLES

Covariant differentials lead to the design of a novel self-tuning power system stabilizer

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Application of differential geometry to study the dynamics of electrical machines by Gabriel Kron evoked only theoretical interest among the power system engineers and was considered hardly suitable for any practical use. Extension of Kron's work led to a physical understanding of the processes governing the small oscillation instability in power system. This in turn has made it possible to design a self-tuning Power System Stabilizer to contain the oscil-

latory instability over an extended range of system and operating conditions. This paper briefly recounts the history of this development and touches upon the essential design features of the stabilizer. It presents some results from simulation studies, laboratory experiments and recently conducted field trials at actual plants – all of which help to establish the efficacy of the proposed stabilizer and corroborate the theoretical findings.

THE application of tensor calculus in analysing the performance of electrical machines was initiated by Gabriel

Kron in the thirties¹. Criticized by physicists for his intrusion into Riemannian geometry, needed for the de-