and is clearly then one of the least active sensitizers in terms of radiation dose. Survival curves for CHO cells with a number of furocoumarins have been published previously[25,30].

A marked difference exists between the formation of SCEs with 3-CPs and DMC. With DMC a typical dose response similar to that seen with furocoumarins[30] was observed (table 2); 3-CPs had considerably less effect and this may be related to its low mutagenic activity and lack of carcinogenicity[9,19].

The parallel behavior of DMC, which cannot form cross-links, is very interesting. DMC causes nearly the same number of SCEs per cell at the same level of cell survival as 5-MOP although considerably more energy was required to produce the same kill (about 8 times as much at LD_{90}, table 3).

DMC is, therefore, a very potent producer of SCEs in contrast to the non-carcinogenic 3-CPs. Angelicin which is only weakly active as a skin photocarcinogen (F. Zajdela; personal communication) produces fewer SCEs per unit lethal dose than the DNA cross-linking linear furocoumarins. Neither compound alone without light, nor light itself, produces cell death.

If DMC should turn out to be a photocarcinogen, and there is no information on this point, then its wide distribution in a number of plants and essential oils would not be without interest.

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Evidence to support the hypothesis that ATP is a co-transmitter in rat vas deferens

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Summary. Application of exogenous ATP or of noradrenaline (NA) produced responses in bisected rat vas deferens which mimicked the biphasic responses to nerve stimulation, and these actions were modified by nifedipine and verapamil in a manner similar to the modification of the 2 phases of the responses of the vas to nerve stimulation. It is proposed that sufficient evidence now exists to support the hypothesis that in this tissue, ATP is released along with NA from the motor nerves and that ATP may indeed be a co-transmitter.

Considerable controversy has surrounded the nature of neurotransmission in the vas deferens of several animal species. There is ample evidence that noradrenaline (NA) is involved[1-3]: however the existence of a 2nd nonadrenergic-noncholinergic (NANC) transmitter is suggested since there is a component of the contractile response to nerve stimulation which is resistant to adrenoceptor blockade or to depletion of NA from the motor nerve terminals. It has been suggested that adenosine triphosphate (ATP), which is present in the storage vesicles of adrenergic nerves may be involved[4,5]. Single pulse electrical field stimulation of the rat vas deferens results in a contraction of the tissue which shows 2 distinct phases, or peaks. The early peak, which occurs 250–280 msec after stimulation, is virtually unaffected by the presence of α-adrenergic blocking agents such as prazosin, or by depletion of neuronal stores of NA, due to prior treatment of the rats with reserpine: the later peak, occurring at 650–700 msec, is reduced or abolished by both

of these procedures, suggesting that this phase is due to the action of NA, released from sympathetic nerves on postsynaptic receptors. We have recently shown that the early phase of the response can be inhibited by concentrations of the calcium antagonist nifedipine ($1\text{-}5\times 10^{-6}$ M) which are without significant effect on the later response[7-9]. However, verapamil, which is also a calcium antagonist potentiates the early phase of the response while blocking the later phase.

Materials and methods. Vasa deferentia from adult Sprague-Dawley rats were divided at a point approximately 60% of the total length measured from the epididymis and both portions were suspended, as previously described[7] in Krebs-Henseleit solution at 38 °C between 2 platinum wire electrodes running parallel to the tissues. The vasa were stimulated at 5-min intervals with a single pulse (1 msec, 300 mA). Contractile responses were measured isometrically, monitored on a storage oscilloscope, and could be permanently recorded on chart paper for further analysis. In some experiments, vasa were taken from rats pretreated with reserpine (5 mg/kg i.p. 18-24 h), or from rats pretreated with 6-hydroxydopamine (6-OHDA), i.v. in 2 separate doses of 100 mg/kg and 250 mg/kg given on the 2 days prior to sacrifice. The effects of NA and ATP were investigated by addition to the tissues in 25 ml organ baths of appropriate volumes of freshly prepared master solutions. The effects of nifedipine and verapamil on the responses to these agents were measured after 15 min contact with the tissues. All experiments involving the use of nifedipine were carried out under sodium light, due to the extreme photolability of this compound. In a few experiments, prostatic ends of the vasa were stimulated with a train of high frequency (500 Hz) pulses containing from 1 to 16 pulses, followed by a single, testing pulse 3 sec after the conditioning train. These experiments were designed to investigate the extent of feedback inhibition of the release of transmitter substances, by examining the size of the response to a single pulse following trains containing varying numbers of pulses, thus releasing varying amounts of transmitter, (French and Scott, in preparation).

Results. Figure 1 shows the effects of nifedipine and verapamil on the biphasic twitch response of both prostatic and epididymal ends of rat vas deferens to stimulation of the intramural nerves by a single pulse of field stimulation. Both phases of the twitch response are seen, the early phase dominant in the prostatic end while the later, adrenergic phase predominates in the epididymal end. While verapamil unexpectedly potentiates the early NANC phase of the contraction in both ends of the tissue, it blocks the later, adrenergic phase. Nifedipine, on the other hand, antagonises the NANC response in both ends of the tissue, and leaves the adrenergic phase largely unaffected. These effects were seen for concentrations of verapamil up to 1×10^{-5} M and up to 1×10^{-5} M for nifedipine and were unaffected by concentrations of prazosin (1×10^{-8} M) sufficient to abolish completely the adrenergic phase of the responses. At concentrations greater than these, both agents caused a reduction in the size of both phases of the response. Figure 2 shows that the actions of these 2 calcium antagonists on the responses to exogenously applied ATP and NA parallel their actions against the 2 phases of the twitch response. Although the effects of only 1 concentration of ATP and 2 for NA are shown, similar actions were demonstrable at both higher and lower concentrations of these 2 agents.

The epididymal end of the tissue is approximately 10 times more sensitive than the prostatic end to the effects of added NA, but is much less sensitive to added ATP. Verapamil antagonises the effects of added NA on both ends of the tissue, paralleling the antagonism of the adrenergic phase of the twitch response; however, this drug potentiates the effects of ATP, which is seen clearly on the more sensitive prostatic end, paralleling its potentiation of the NANC response to nerve stimulation. Nifedipine, on the other hand, exhibits only slight antagonism towards the responses to exogenous NA, but causes a marked reduction in the responses to ATP; these actions parallel the lack of antagonistic activity towards the adrenergic phase of the twitch response, and the marked inhibition of the NANC response.

Prostatic ends of the vas deferens responded to high frequency (500 Hz) trains of pulses with a twitch response which was directly related to the number of pulses in the train. Delivery of a single 'testing' pulse 3 sec after the 'conditioning' pulses of the train also resulted in a single twitch response. The size of this response decreased as the number of pulses in the train increased, suggesting autoinhibition of the release of the transmitter in response to the testing pulse. The response to the testing pulse was reduced by nifedipine and peaked at around 250-280 msec, suggesting that the transmitter responsible was not NA.

In prostatic ends of vasa taken from reserpine treated rats the responses to trains of pulses were also directly related to the number of pulses in the train but the response to a testing pulse applied 3 sec later was independent of the size of the response to the train. This suggests the absence of any feedback regulation of the output of the transmitter. The responses to both the trains and the single, testing pulses were abolished by nifedipine.

Discussion. It is suggested that these results, taken together with results of other studies, satisfy the criteria for the identification of a substance as a putative transmitter[10]: viz.

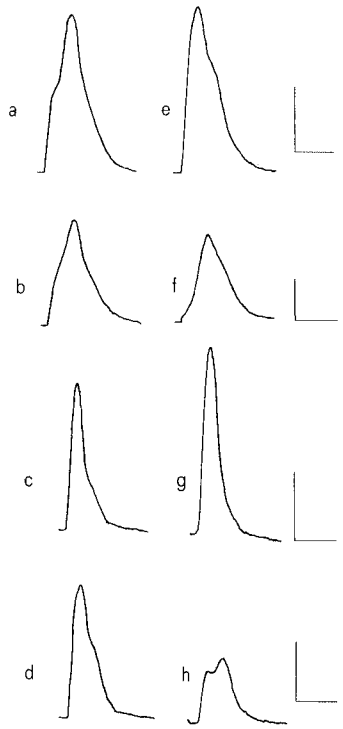

Figure 1. The effects of verapamil and nifedipine on the twitch responses of the rat vas deferens to single pulse stimulation; control responses from epididymal ends (a and b) and prostatic ends (c and d) of the tissue. The effects of verapamil (5×10^{-6} M) are shown in e and g, and of nifedipine, (5×10^{-7} M) in f and h. Each pair of responses is from separate tissues: vertical calibration bars, 1 g; horizontal bars, 1 sec throughout.

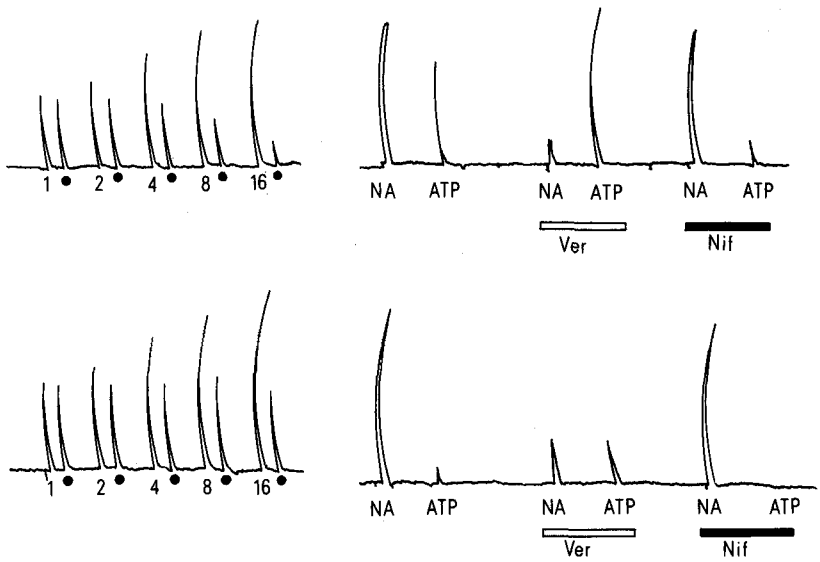

Figure 2. Left side. Responses of prostatic ends of rat vas deferens to trains of pulses (500 Hz, 300 mA, 0.3 msec) containing the number of pulses indicated, and to a single testing pulse (300 mA, 3 msec, at ●) delivered 3 sec later. The chart was switched off during the 5 min between each train. Upper trace from untreated rat, lower trace from reserpinised rat. Vertical bar, 1 g. Right side. The effects of verapamil and nifedipine on the contractile actions of noradrenaline (NA) and adenosine triphosphate (ATP) in rat vas deferens. Top trace prostatic end; NA 5×10^{-5} M, ATP 2×10^{-4} M throughout. Ver indicates verapamil (1×10^{-5} M), Nif indicates nifedipine (5×10^{-8} M). 2nd trace epididymal end; NA 5×10^{-6} M; ATP, verapamil and nifedipine concentrations as above. All responses were recorded isotonically at 5-min intervals; the chart was stopped between drug additions.

a) ATP has been shown to be present in the small dense cored vesicles of the terminal varicosities of adrenergic nerves[11-13]. b) The release of ATP along with NA has been demonstrated in the hypogastric nerves supplying the vas deferens[11]. c) The responses to nerve stimulation and to application of exogenous ATP are short lasting; this suggests a rapid removal or breakdown of the transmitter. Burnstock[5] has suggested that released ATP is rapidly broken down by Mg-activated ATPase and 5′-nucleotidase to adenosine. The adenosine may then be taken up by the terminal varicosities, converted to ATP and then reincorporated into the storage vesicles. Adenosine not taken up by this process may be deaminated to pharmacologically inactive inosine by adenosine deaminase. d) The results presented here indicate that ATP, exogenously applied to the vas deferens, has the same effect as nerve stimulation, viz a brief contraction, more prominent in the prostatic end of the tissue. e) The action of verapamil and nifedipine on the responses of the vas to exogenously applied ATP and NA are identical to their actions on the early and late phase respectively of contractions elicited by single pulse field stimulation. f) Aberer et al.[12] have shown that ATP can be taken up from the cytoplasm of adrenergic nerves into the small dense cored vesicles by a carrier mediated process. Regulatory control of transmitter release in the rat vas deferens would appear to be a property of the NA component, since feedback inhibition can be demonstrated only in tissues with a replete NA content. Depletion of the NA content by pretreatment of the rats with reserpine does not abolish the responses to nerve stimulation, which are presumably maintained by ATP release, but the independence of the size of the response to a testing pulse after trains of varying length would indicate that ATP does not 'feed back' on to presynaptic purinergic receptors. Pretreatment of the rats with 6-OHDA by the i.v. route markedly reduced both phases of the contractile responses to nerve stimulation. This result confirms the findings of Fedan et al.[14] but is at variance with other workers[15] who suggested that the NANC response was still present in vasa taken from 6-OHDA treated rats. We believe that the difference lies in the route of administration and the dose given which was higher in our experiments and those of Fedan et al. The absence of both phases of the response suggests the possibility that a 2nd transmitter might indeed be released, along with NA, from the same nerve endings. Since NA is complexed with ATP in the vesicles of sympathetic nerves[16,17], and the release of both substances from hypogastric nerves has been demonstrated[11], the possibility exists that ATP is the agent responsible for the NANC phase of the twitch response, and may therefore be considered to be a co-transmitter.

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