*Br. J. Pharmac.* (1981), **73**, 321–323

# A COMPARISON OF THE EFFECTS OF NIFEDIPINE AND VERAPAMIL ON RAT VAS DEFERENS

A.M. FRENCH & N.C. SCOTT

Pharmacology Section, Department of Pharmacy, Heriot-Watt University, Edinburgh

Nifedipine preferentially blocks contractions of the prostatic end of the rat vas deferens to single pulse field stimulation, leaving the epididymal end largely unaffected. This action is not due entirely to antagonism of calcium influx. Verapamil unexpectedly potentiated the responses of the prostatic portion, and antagonized those of the epididymal end. The use of nifedipine may, therefore, allow investigations of adrenergic mechanisms on this tissue to be studied without the complications of non-adrenergic transmission.

**Introduction** In rat vas deferens, transmission may involve noradrenergic mechanisms, especially in the epididymal end and a non-adrenergic component in the prostatic portion (Anton, Duncan & McGrath, 1977; McGrath, 1978). The non-adrenergic transmitter is unknown, and selective inhibition of contractions of the prostatic portion of the tissue has not been demonstrated. This paper suggests that the calcium antagonist nifedipine, but not verapamil, preferentially inhibits contractions of the vas due to this 'unknown' transmitter. The use of nifedipine thus allows a study of adrenergic mechanisms in the vas deferens to be performed without the complications of the non-adrenergic component.

**Methods** Vasa deferentia from mature male rats (250–300 g) were bisected transversely so that the epididymal end was 60% and the remaining (prostatic) portion was 40% of the total length as described by McGrath (1978). Both portions were suspended between two vertical platinum wire electrodes situated 1 cm apart in a tissue bath containing 100 ml of Krebs-Henseleit solution of the following composition (mM): NaCl 118, KCl 5.4, $CaCl_2$ 2.52, $MgSO_4$ 1.18, $KH_2PO_4$ 1.1, $NaHCO_3$ 25 and glucose 11.1. When aerated with a mixture of 95% $O_2$ and 5% $CO_2$, the pH was 7.4 at 37°C. The tissues were stimulated at 5 min intervals with single (1 ms) pulses at supramaximal voltage until the responses became constant (usually about 20 min). Using these parameters, tetrodotoxin ($5 \times 10^{-8}$M) completely inhibited the responses, confirming their nervous origin. The twitch responses of both portions were measured with strain gauges and displayed on a Storage Oscilloscope. Permanent recordings were made on a chart recorder connected to the oscilloscope via a digital-analogue output converter. Tissues were maintained at a resting tension of 0.5 g by a servo-mechanism supporting the transducers. Drug solutions were added to the bath cumulatively and the heights of the twitch responses measured after 10 min contact with each concentration, to permit equilibration. When nifedipine was used, the bath and solutions were protected from light.

The drugs used were nifedipine (Bayer, A.G.) and verapamil (Abbott).

**Results** The prostatic portion of the vas deferens is much more sensitive than the epididymal end to inhibition by nifedipine. Nifedipine ($3 \times 10^{-6}$M) reduced twitch height in the prostatic portion by 95% but only by 20% in the epididymal end (Figure 1).

While verapamil also antagonized the responses of the epididymal portion, those of the prostatic end were potentiated, at concentrations up to $1 \times 10^{-5}$M. The highest concentration used ($5 \times 10^{-5}$M) caused less potentiation of the prostatic twitch height than $1 \times 10^{-5}$M.

When the calcium concentration of the bathing medium was increased from 2.52 mM to 3.76 mM, as expected, the effects of nifedipine were antagonized. At $1 \times 10^{-6}$M, nifedipine reduced prostatic twitches by $82 \pm 2\%$ in 2.52 mM calcium, but only by $49 \pm 4\%$ in 3.76 mM calcium ($P < 0.001$, $n = 6$). The same concentration of nifedipine reduced epididymal contractions by $18 \pm 3\%$ in 2.52 mM calcium, but only by $5 \pm 3\%$ in 3.76 mM calcium ($P < 0.01$, $n = 6$). However, lowering the calcium concentration to 1.76 mM also antagonized the effects of nifedipine (prostatic twitches reduced by only $50 \pm 4\%$ ($P < 0.001$, $n = 6$), and epididymal twitches by $10 \pm 2\%$ ($P < 0.02$, $n = 6$)). By contrast, the effects of verapamil in potentiating the prostatic or reducing the epididymal responses, were not significantly altered by changes in the calcium concentration between 1.26 mM and 4.26 mM.

The calcium-dependence of the size of the twitch responses of each portion of the vas was determined by measuring the responses to single pulses after equilibration in the presence of calcium concentrations ranging from 1.26 mM to 4.26 mM. At calcium

0007-1188/81/060321-003 $01.00

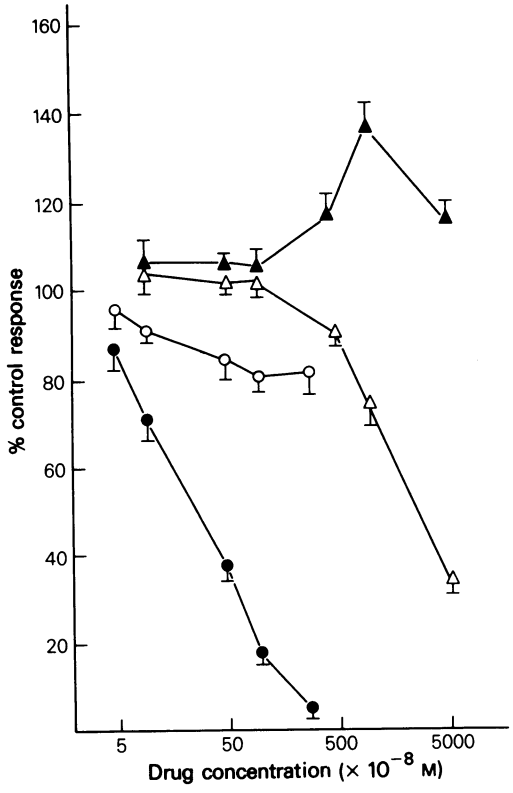


**Figure 1** Responses of epididymal and prostatic portions of rat vas deferens to single pulse field stimulation in the presence of nifedipine (● prostatic, ○ epididymal) and verapamil (▲ prostatic, △ epididymal). Each point is the mean response of six tissues, expressed as a percentage of the control (pre-drug) response: vertical bars are s.e.mean.

concentrations below 1 mM, the twitch responses of both portions were too small to be measured accurately; increasing the calcium concentration above 4.5 mM caused precipitation of the calcium from solution. At 'high' concentrations of calcium (i.e. above 2.52 mM) the twitch responses of both portions of the tissue were potentiated equally, e.g. at 3.26 mM calcium the epididymal portion twitches were potentiated by 26±4%, while the prostatic twitches were potentiated by 31±4% ($P>0.5$, $n=6$). Reduction of external calcium concentration from 2.52 mM caused a reduction in the twitch height of both portions, although the epididymal portion did appear to be the more affected, e.g. at 1.76 mM calcium the response of the epididymal portion was reduced by 42±5%, the corresponding reduction of the prostatic portion being 31±2% ($P<0.05$, $n=6$). Similarly at 1.26 mM calcium the epididymal responses were down by 78±3%, and the prostatic responses were reduced by 59±3% ($P<0.01$, $n=6$).

**Discussion** The results show that the non-adrenergic response in the prostatic portion of the rat vas deferens can be inhibited preferentially. This contraction is resistant to α-adrenergic blockade, and reserpine pretreatment while the responses of the epididymal end of the tissue are reduced greatly or abolished by such treatments (McGrath, 1978). The technique of single pulse stimulation should preclude any involvement of negative feedback of transmitter release, whether this be adrenergic or non-adrenergic.

The preferential antagonism of the prostatic twitch responses by nifedipine may be greater than the results suggest. The apparent 20% reduction in the responses of the epididymal end caused by $3\times10^{-6}$ M nifedipine, compared with the 95% reduction in the responses of the prostatic portion, may be due to the fact that each bisected portion of the vas contains a small proportion of activity of the other portion. Since the prostatic twitch peak height occurs at 250 ms and the epididymal twitch peak at 650 ms, under normal conditions, mechanical interaction may cause the epididymal twitch to 'take-off' from an increased 'baseline' due to the initial twitch of the prostatic element in the epididymal portion. Thus in the presence of concentrations of nifedipine that inhibit prostatic twitches, the epididymal twitch may be also reduced by inhibition of the prostatic element.

Low calcium concentrations depress and increased calcium increases the contractions to single pulse stimulation in both portions of the vas. However, the preferential blocking action of nifedipine on the prostatic portion was antagonized both by decreasing and by increasing the external calcium concentration suggesting that antagonism of calcium influx may not be involved. Also, the calcium antagonist verapamil (Fleckenstein, 1977) which also inhibits slow sodium channels (Shigenobu, Scheider & Sperelakis, 1974) potentiates the twitch responses of the prostatic potion of rat vas, an effect unaltered by changes in the external calcium ion concentration.

In conclusion, while it is possible to expose the non-adrenergic component of transmission in rat vas deferens by the use of postsynaptic adrenoceptor antagonists or by reserpine pretreatment, preferential inhibition of the non-adrenergic component has not been successfully achieved to date. Nifedipine should fulfil this purpose and allow examination of adrenergic mechanisms without the complicating non-adrenergic component.

## References

ANTON, P.G., DUNCAN, M.E. & MCGRATH, J.C. (1977). An analysis of the anatomical basis for the mechanical response to motor nerve stimulation of the rat vas deferens. *J. Physiol.*, **273**, 23–43.

FLECKENSTEIN, A. (1977). Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. *A. Rev. Pharmac. Tox.*, **17**, 149–166.

MCGRATH, J.C. (1978). Adrenergic and 'non-adrenergic' components in the contractile response of the vas deferens to a single indirect stimulus. *J. Physiol.*, **283**, 23–39.

SHIGENOBU, K., SCHNEIDER, J.A. & SPERELAKIS, N. (1974). Verapamil blockade of slow $Na^+$ and $Ca^{++}$ responses in myocardial cells. *J. Pharmac. exp. Ther.*, **190**, 280–288.

*(Received March 11, 1981.)*