

# 11

## EXPERIMENT ELEVEN

# Rat Vas Deferens

The rat vas deferens is rich in sympathetic innervation, and is useful in showing the effects of  $\alpha$ -adrenoreceptor agonists and antagonists, and a range of drugs that presynaptically inhibit neurotransmission.

This preparation is commonly used to examine the pharmacology of  $\alpha$ -adrenoreceptors, and to demonstrate indirect agonist action (by tyramine). Each vas deferens can be cut into epididymal and prostatic parts, for unstimulated and stimulated experiments respectively.

### Dissection

Each rat vas deferens is dissected out and cut into two halves: the epididymal end (closest to the testis; a small amount of the tissue is intratesticular) and the prostatic end (closest to the urethra). Take care not to tug or stretch the tissue. Removal of the vas with the cauda epididymis intact assists the visual identification of the epididymal and prostatic ends. If the length of vas needs to be reduced, trim the prostatic end. Plate 18 shows dissection of a male rat, with the vas deferens visible.

The epididymal half (unstimulated preparation) is attached to a tissue holder at one end and to a force transducer at the other end. The preparation is set up under 1 g wt tension and allowed to equilibrate for 20–30 minutes.

The prostatic half of the vas deferens (stimulated preparation) is tied over a stimulating electrode. A tie attaches the upper end to a force transducer. The second electrode is arranged as near to the tissue as possible without impeding its movement. Alternatively, the tissue can be threaded through loosely-fitting ring electrodes. A tension of 1 g wt is applied and the tissue allowed to equilibrate for 20–30 minutes.

### Experiment – Epididymal Half

The epididymal half of the vas deferens is unstimulated.

#### Chart Settings

This is a single-channel arrangement with a PowerLab, Bridge Amp, and force transducer. In Chart, the range should be chosen to suit the maximum force to be exerted on the transducer. The final scale after units

conversion and so on should be  $-0.5$  to  $5$  g wt. The sampling rate should be at least  $100$  samples/min. The view compression should be  $10:1$ . A high-pass filter of  $10$  Hz should be chosen in the Bridge Amplifier dialog box. Data Pad miniwindows can be set up to show the comment number and text, and the mean of the selection or value at the active point (using the Mean function).

## Protocol

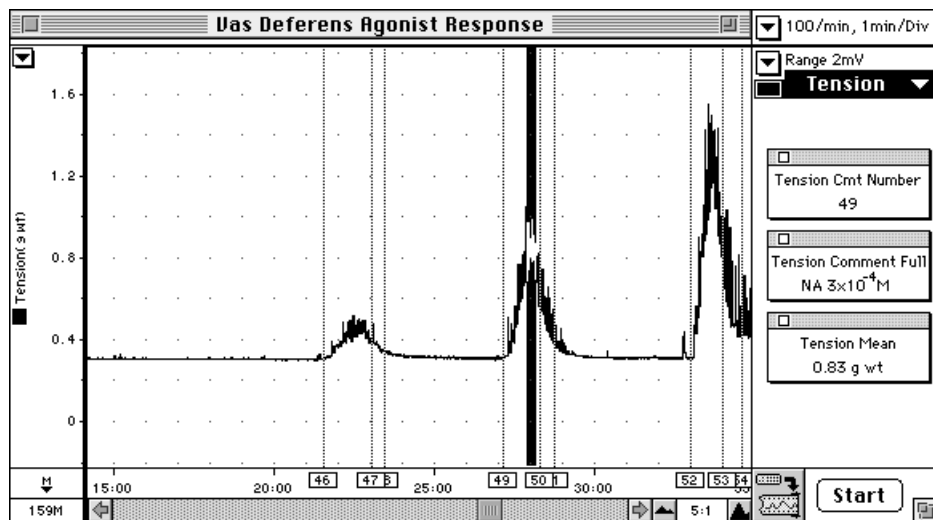
Agonists such as noradrenaline or phenylephrine, and indirect agonists such as tyramine, contract the tissue, usually with an increase in 'spontaneous' twitch-like activity (Figure 11-1). In quantitating these responses, it is convenient to average out the spontaneous variations, by selecting several seconds of data and reading the response value in a miniwindow that shows the mean.

In determining a dose-response curve for noradrenaline, suggested concentrations are:  $10^{-8}$  M,  $10^{-7}$  M,  $3 \times 10^{-7}$  M,  $10^{-6}$  M,  $3 \times 10^{-6}$  M,  $10^{-5}$  M,  $3 \times 10^{-5}$  M,  $10^{-4}$  M. The higher concentrations may need two washes. Antagonism by an  $\alpha$ -adrenoreceptor blocker may be shown with phentolamine ( $5 \times 10^{-5}$  M) or prazosin ( $10^{-6}$  M).

Figure 11-1 shows the response of rat vas deferens to noradrenaline in the presence of the alpha-blocker prazosin. Parameters from the selection made over the peak response are shown in the miniwindows at right.

**Figure 11-1**

Response of rat vas deferens to noradrenaline  $10^{-4}$  M,  $3 \times 10^{-4}$  M, and  $10^{-3}$  M in the presence of the alpha-blocker prazosin.



## Experiment – Prostatic Half

The prostatic half of the vas deferens is stimulated.

### Chart Settings

This is a single-channel arrangement with a PowerLab, Bridge Amp, and force transducer. In Chart, the range should be chosen to suit the maximum force to be exerted on the transducer. The final scale after units conversion and so on should be  $-0.5$  to  $5$  g wt. The sampling rate should be

at least 40/s (the relatively high rate is needed to ensure accuracy in recording twitch contractions). The view compression should be 50:1. A high-pass filter of 10 or 20 Hz should be chosen in the Bridge Amplifier dialog box. Data Pad miniwindows can be set up to show the comment number and text, and the mean of the selection or value at the active point (using the Mean function).

## Stimulation

Field stimulation with loosely fitting electrodes is inefficient, so that quite strong stimuli may be needed to activate autonomic nerves in the vas deferens. Adjust the stimulus strength for reproducible maximal twitch contractions in response to 0.3 ms pulses. This typically requires 20–60 V; if necessary, increase the stimulus width for stronger stimulation. Set the stimulator for continuous stimulation at 0.2 Hz.

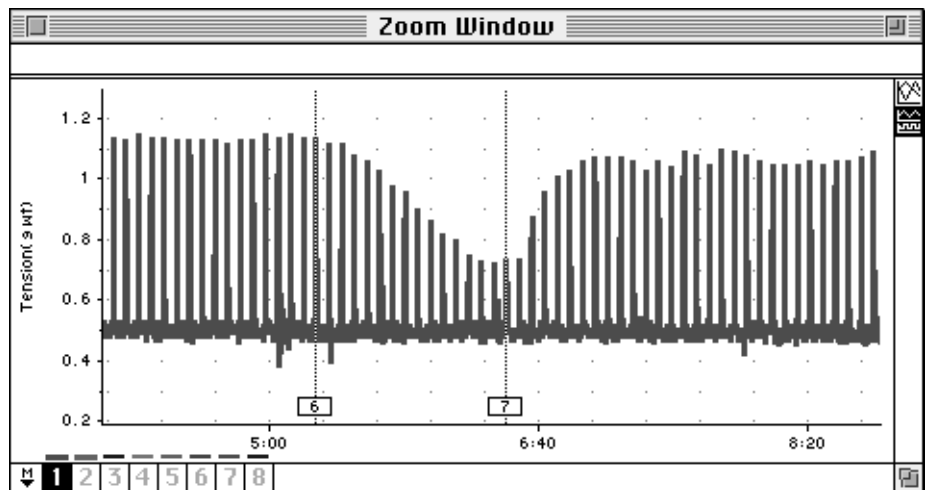
## Protocol

With stimulation inducing twitch responses in the rat vas deferens,  $\alpha_2$ -mediated presynaptic inhibition of the twitch can be shown with clonidine ( $10^{-8}$  M). The inhibition is reversed by idazoxan ( $3 \times 10^{-7}$  M). Opioid agonists (morphine, enkephalins) also inhibit the twitch, this effect being blocked by naloxone.

Figure 11–2 shows a zoomed view of stimulated twitch responses in the rat vas deferens, illustrating the inhibitory effect of clonidine and its reversal by the imidazoline receptor-blocker idazoxan.

**Figure 11–2**

Stimulated twitch responses in rat vas deferens. Clonidine  $10^{-8}$  M is added at comment #6, and its inhibitory effect is reversed by the imidazoline receptor-blocker idazoxan  $3 \times 10^{-7}$  M added at comment #7.



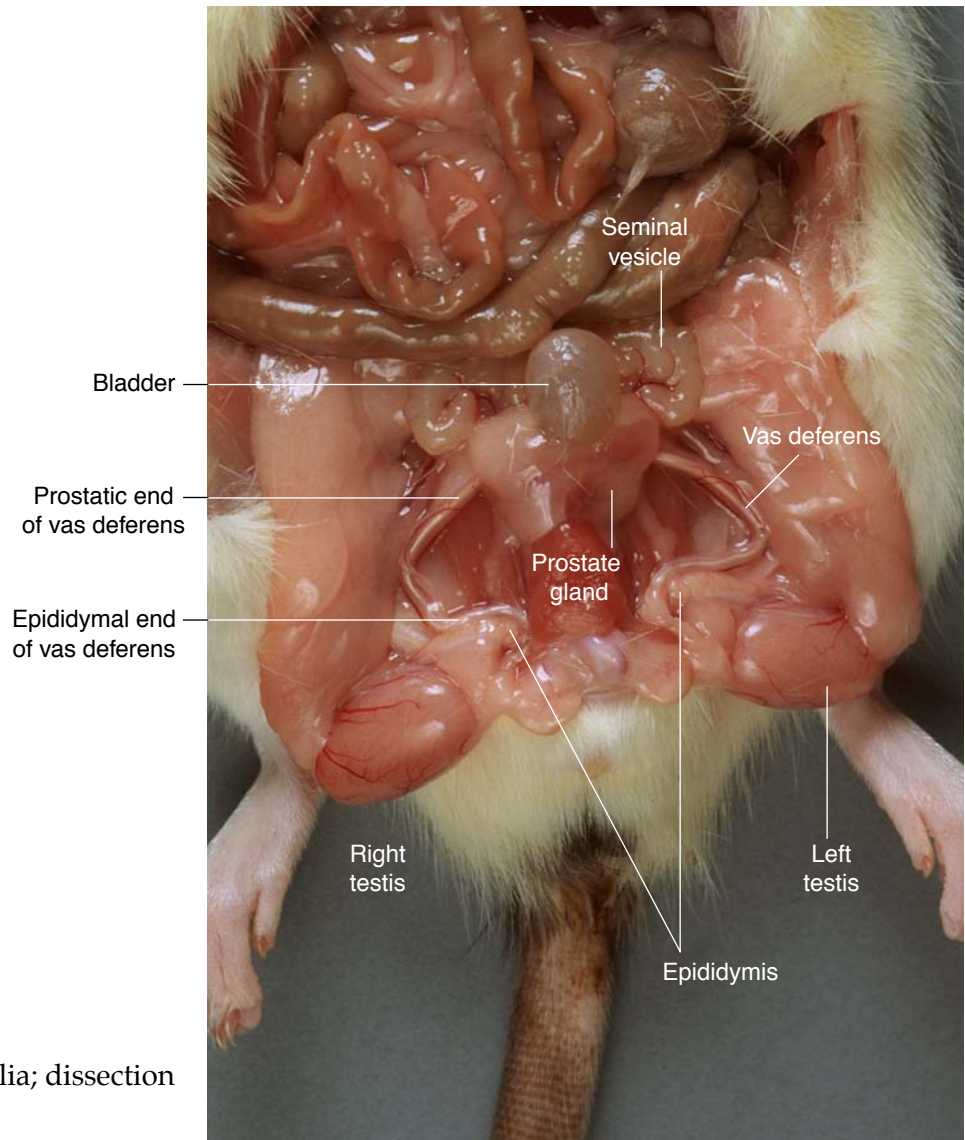
## Further Work

It is possible to use mouse or guinea pig instead of rat. There is considerable species variation in receptors and the nature of drug responses. The vas deferens has purinoceptors<sup>1</sup> as well as adrenoceptors, and can be used to examine the effects of purinergic agonists and antagonists.

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## Reference

1. B.S. Khakh, A. Surprenant, and P.P.A. Humphrey, A study on P2X purinoceptors mediating the electrophysiological and contractile effects of purine nucleotides in rat vas deferens, *British Journal of Pharmacology* 115: 177–185 (1995).



**Plate 18.** Male rat genitalia; dissection of vas deferens in situ.