D1

Measurement of renal sympathetic nerve activity in freely moving mice

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A large number of genetically modified mice are now available that can be used to elucidate the role of the deleted, mutated, or over-expressed genes for regulation of physiological functions. The impact of anaesthesia on physiological parameters in the mouse is such that it could overshadow subtle changes induced by gene manipulation. Therefore, many approaches have been attempted to measure physiological parameters in conscious mice (Janssen and Smits, 2002). A major control mechanism is the sympathetic nervous system, yet there have been few reports of its successful measurement in the conscious mouse. In the present study, we report a method to reliably measure renal sympathetic nerve activity in freely moving mice.

C57BL/6J mice (n=4), weighing over ~ 25g, were used for all experiments. Procedures were undertaken in accordance with National guidelines. Mice were anaesthetized with pentobarbital sodium (45 mg Kg^{-1} I.P.). Electrodes were implanted for the measurement of renal sympathetic nerve activity (RSNA), using an adaption of the method employed in rats (Miki et al. 2002). Briefly, the left kidney was exposed retroperitoneally. The sympathetic nerves running alongside the renal artery or vein were carefully dissected free of connective tissue. A piece of laboratory film (~1 mm x 2 mm) was placed under the dissected nerve. The two tips of the electrodes were hooked onto the nerve by placing the electrodes between the renal nerve and the sheet. The exposed nerve and the electrode were embedded in a two-component silicone gel (932, Wacher-Chemie, Munich, Germany). Subsequently, the electrode for the measurement of the electrocardiogram (ECG) was also implanted. After 2 days of recovery, recordings were carried out in a sound attenuated, temperature (24°C) controlled chamber. Animals were killed with an anaesthetic overdose. Data (means ± S.E.M.) were subjected to the Fisher's least significant difference test and significance taken as $P<0.05$.

The renal sympathetic nerve activity was recorded successfully without contamination by external noise such as that from the electrocardiogram and/or electromyogram. The renal sympathetic nerve activity was increased to 299 ± 12 % ($P<0.05$) during movement from 100% of the non-REM and quiet awake state and heart rate was concomitantly increased from 576 ± 3 beats min^{-1} of the non-REM sleep and quiet awake level to 706 ± 3 beats min^{-1} ($P<0.05$). Successful recordings could be made for up to 5 days. This novel method for measurement of the RSNA could be usefully employed in studies on the role of sympathetic nerve activity in regulating physiological function in genetically modified mice.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

D2

AN ANAESTHETISED RAT MODEL FOR STUDYING THE CENTRAL NERVOUS CONTROL OF SYMPATHETIC CUTANEOUS VASOCONSTRICTOR ACTIVITY REGULATING A THERMOREGULATORY CIRCULATION

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There are relatively few studies where the central nervous control of cutaneous vasocontrictor (cvc) tone has been analyzed by recording sympathetic activity. This is probably because time consuming fibre picking techniques, lacking long term stability, have been used to sample cvc activity (Habler et al., 2000). Here we present a simple technique, providing stable recordings, that has been used successfully in our laboratory to sample population sympathetic activity regulating the rat tail (thermoregulatory) circulation.

Urethane-anaesthetised male rats (urethane, initial dose, 1.3 g kg^{-1}, I.P., supplemented with 5-10 mg I.V. as required) are positive pressure ventilated with oxygen-enriched air. A femoral artery and vein are canulated to monitor blood pressure and to administer drugs, respectively.

Rats have their cauda equina transected to remove the somatomotor component of nerve activity from tail nerves (collector nerves) (Smith & Gilbey, 1998). Monophasic sympathetic activity is recorded from a dorsal or ventral collector nerve using implanted bipolar silver wire /suction electrodes (Johnson & Gilbey, 1994; Korsak & Gilbey, 2004). Rats are humanely killed at the end of experiments with a urethane overdose (200 mg I.V., in a bolus injection).

This technique has been used for exploring the medullary control of cutaneous activity (Korsak & Gilbey, 2004) and here we focus on our current studies exploring the spinal circuitry and the mechanisms involved in the generation of rhythmical discharges in cvc activity.

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