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Sadananda P[1], Drake M[2], Paton J[1], Pickering A[1]
1. *University of Bristol,* ***2.*** *Bristol Urological Institute*

A DECEREBRATE, ARTERIALLY PERFUSED IN SITU PREPARATION OF RAT FOR THE STUDY OF AUTONOMIC CONTROL OF MICTURITION

Hypothesis / aims of study

Bladder autonomic research has been impeded by the difficulty of establishing whole animal models, that permit the simultaneous investigation of central and peripheral neural activity during bladder filling and voiding. The aim of this study was to extend an established arterially perfused in situ decerebrate rat preparation [1] to characterise micturition by recording bladder pressure and external urinary sphincter (EUS) EMG. It was hypothesised that this in situ preparation will yield a physiologically viable and robust approach to allow investigation of the autonomic control of micturition.

Study design, materials and methods

Female Wistar rats (40-80g) were anesthetized with halothane. Access to the bladder was achieved via a midline laparotomy, and the stomach, spleen and intestine were tied off and removed. The heart was accessed via a midline sternotomy. The animal was immediately immersed in cold artificial CSF (aCSF) and decerebrated, at which point the anaesthetic was withdrawn. The preparation was placed in a recording chamber and a double lumen cannula was inserted into the ascending aorta via an incision at the apex of the heart. The preparation was arterially-perfused with carbogen-gassed, aCSF (32ºC). The heart resumed beating immediately once perfusion commenced. Rhythmic respiratory muscle contractions were seen within minutes, as perfusion pressure reached 40-60mmHg.

A glass suction electrode was used to record activity from the phrenic nerve, which was used as a physiological indicator of preparation viability. A cannula (i.d.200µm) was inserted into the left ureter for bladder filling (10µl/min with artificial urine). A second cannula was inserted into the bladder dome for pressure monitoring. Midline dissection of the pubis symphysis revealed the EUS. A bipolar glass suction electrode (i.d.0.5mm) was positioned laterally on the proximal sphincter to record EUS EMG. A camera microscope allowed synchronous monitoring of bladder contractions and the precise times of fluid ejection from the urethra.

Results

The technique produces viable preparations with robust eupnoeic phrenic activity, and cardiorespiratory reflexes indicative of an intact brainstem. It takes approximately 30min to set up the preparation and obtain phrenic nerve recording. Insertion of bladder filling and pressure cannulae, and dissection of the pubic symphysis takes a further 10-15min.

Out of 26 rats, spontaneous bladder contractions (SBC) were seen in 23 preparations. The average frequency and amplitude of these contractions was 4.2 ± 1.1/min and 3.8 ± 2.0mmHg, respectively (n=10). The SBCs were seen to follow waves of contraction propagated down the ureter to the bladder. Infusion of fluid into the bladder increased the frequency of occurrence of the SBCs. The SBCs did not typically result in urination indicating intact lower urinary sphincter control. Bladder pressure recordings during fluid infusion showed the characteristic three phases (Fig 1) – rise in intraluminal pressure, high frequency oscillation of intraluminal pressure, followed by a sharp decline - as described originally in rat [2]. This pattern was compromised in preparations that had lost their cardiorespiratory rhythm.

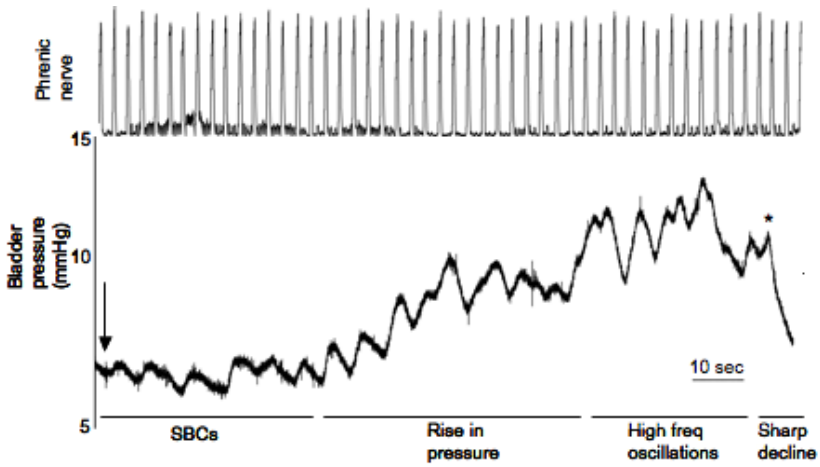

Figure 1. Robust and stable phrenic nerve activity indicating viable brainstem function, during fluid infusion into the bladder. (arrow indicates start of infusion; * indicates void)

EUS EMG activity comprised of bursting activity. In three of the nine preparations, strong bursting activity in the sphincter EMG pre-ceeded and synchronised with the SBCs (Fig 2). Application of a muscle relaxant, vecuronium bromide (2µg/ml), abolished the EMG response completely. Ganglion blocker, hexamethonium (300uM), reduced EUS EMG activity. Both agents resulted in leakage of urine, associated with each SBC.

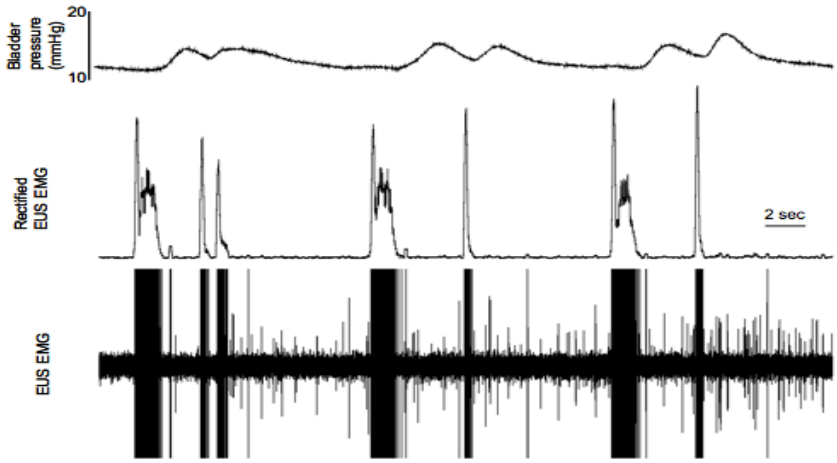

Figure 2. Multi unit EMG activity pre-ceeding each SBC.

Interpretation of results

The present in situ decerebrate rat model provides reliable preparations with robust brainstem control. Autonomic control of micturition is also intact, as seen by strong EUS EMG activity and SBCs, similar to those seen in anesthetised rats. SBCs have previously been considered to be distension related [3]. This was confirmed in the present study, where SBCs were initiated after bladder filling. The pre-ceeding EUS EMG activity is suggestive of excitatory signalling to the EUS to maintain contraction and prevent urine leakage during each SBC. Application vecuronium bromide abolished the EMG response completely, confirming that the recording was from EUS. In addition, the reduced EUS EMG activity after administration of hexamethonium was in line with leakage of urine associated with each SBC.

Concluding message

The present decerebrate artificially perfused in situ rat preparation has been successfully applied to study autonomic control of micturition. This technique overcomes restrictions present in vivo, such as the need for anaesthesia, previously shown to interfere with micturition. In addition, the bloodless working field aids visualisation. Manipulation of the extracellular environment can be achieved with relative ease. This technique can be extended to record activity from nerve bundles innervating the bladder and sphincter as well as to study acute and chronic pathological models.

References

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