PS C73

The expression of cation–chloride cotransporters in the rat brain: regulation of KCC2 in glucose-sensing regions of the hypothalamus

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The cation–chloride cotransporter (CCC) family comprises seven known Na^+–Cl^- (NCC), Na^+–K^+–$2Cl^-$ (NKCC) and K^+–Cl^- (KCC) cotransporters. Neurones expressing KCC have low intracellular Cl^- such that GABA-gated Cl^- channel activation results in Cl^- influx, generating an outward (inhibitory) current. In contrast, Cl^- channel activation in cells expressing NKCC (or NCC), which maintain high intracellular [Cl^-], will generate an inward current due to Cl^- efflux. We have evidence that the regulation by glucose of pancreatic islet cell function involves activation of an anion channel (Best, 2002), and that differential expression of CCCs determines whether glucose is stimulatory (β-cell) or inhibitory (α-cell). It is likely that glucose-sensing neurones utilise mechanisms similar to those in islet cells. We have therefore studied the distribution of known CCCs by RT-PCR.

The animals used for the experiments were adult male Sprague-Dawley rats, which were humanely killed. KCC1, KCC3a and KCC4 were expressed in all the tissues tested (cerebellum, hypothalamus, kidney, liver and pancreas). Other CCCs had a more limited distribution: NKCC1 in the brain (and more weakly in the kidney and pancreas); NKCC2 in the kidney only; NCC1 and KCC3b in the kidney and pancreas; KCC2 in the brain only. Interestingly, for KCC3a, two amplification products were found in the hypothalamus, cerebellum, and other areas of the brain.

In situ hybridisation of rat forebrain sections demonstrated that while the expression of NKCC1 and KCC1 was restricted to the choroid plexus, KCC3a was expressed in the hippocampus and piriform cortex. KCC2 was widely expressed in the brain, but with higher concentrations in the piriform cortex, hippocampus, thalamus, the lateral hypothalamic area (LHA) and the ventromedial hypothalamic nucleus (VMH). KCC4 was present in choroid plexus and in the suprachiasmatic nucleus of the hypothalamus. Since the LHA and VMH are regions known to be involved in energy homeostasis we examined the expression of KCC2 in rats fasted for 48 h ($n = 12$). There was a significant decrease in KCC2 mRNA levels following fasting in the VMH and LHA (32 %, $P < 0.05$ and 69 %, $P < 0.001$; Student's unpaired t test), but no significant change in the thalamus. There were no differences in KCC4 in fed and fasted rats. Since the LHA and VMH areas of the brain are rich in glucose-inhibited neurones, regulation of KCC2 by fasting would be consistent with a role in glucose sensing. However, the change in KCC2 may reflect altered functioning of GABA receptors related to energy balance. In contrast, there was no evidence for the predicted expression of either known NKCC isoform in the VMH, which is also rich in glucose-stimulated neurones.

Best L (2002). *Diabetes Metab* **28**, 3S18–3S24.

This work was funded by the BBSRC.

All procedures accord with current UK legislation

PS C74

Identification of the novel Ca^{2+}-activated Cl^- channel (mCLCA3) in adult murine brain

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Recently a novel family of putative calcium-activated chloride channel (CLCA) proteins from bovine, murine and human origin have been identified by several laboratories (Gruber *et al.* 2000). We have previously reported the distribution of one of these homologues, the mCLCA3 protein, in peripheral tissues of the adult mouse (Shenton *et al.* 2002; Winpenny *et al.* 2002). However, very little is known about the role of this channel family in the mammalian brain. Here we provide the first evidence for the existence of the mCLCA3 channel in the adult mouse brain.

Our novel affinity-purified anti-mCLCA3 was generated in rabbits to a peptide sequence corresponding to the sequence KLETFKNAD (95–103) from the mCLCA3 amino acid sequence (GenBank Accession No. AB017156), following previously successful strategies (Chazot *et al.* 1994). All animals were humanely killed. The antibody was used to map the cellular distribution of the mCLCA3 channel protein in the adult murine brain using standard immunoblotting and immunohistochemical techniques (Thompson *et al.* 2002).

Initially, RT-PCR was performed using selective mCLCA3 oligonucleotide primers. The reaction identified a robust mRNA species for mCLCA3 in both adult mouse forebrain and cerebellum.

Immunoblotting experiments on mouse forebrain and cerebellar tissue homogenates detected major immunoreactive species present at 200 kDa, 120 kDa and 70 kDa. All these bands were blocked by prior incubation of the anti-mCLCA3 antibody with the immunising peptide. Immunohistochemical staining was carried out using Vectastain ABC kit according to manufacturer's instructions together with anti-mCLCA3 as primary antibody. This technique demonstrated widespread neuronal labelling in many key structures within the brain, including the hippocampal formation and striatum and there was modest cortical and cerebellar staining. Immunoreactivity was negligible after preadsorption of the anti-mCLCA3 antibody with the immunising peptide. Highest expression was evident in the hippocampal formation with robust immunoreactive labelling of the cell bodies of the pyramidal neurons, granule cells and some hilar cells of the dentate gyrus.

Together these data demonstrate the expression of the mCLCA3 channel protein in neuronal tissue and suggest that the mCLCA3 channel may play a role in distinct populations of neurons and/or glia in the mammalian CNS.

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Shenton FC *et al.* (2002). *Pediatr Pulmon Suppl* **24**, 243.

Thompson CL *et al.* (2002). *Mol Brain Res* **102**, 55–61.

Winpenny JP *et al.* (2002). *J Physiol* **539.P**, 2*P*.

This work was supported by the Wellcome Trust (059897) to J.P.W. & P.L.C.

All procedures accord with current UK legislation

PS C75

Hypoxia selectively modulates ligand-gated P2X receptor cation channels

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In the nervous system purinergic excitatory synapses use ATP to mediate fast synaptic transmission via activation of P2X receptor cation channels. In sensory neurons the ATP-induced current is mediated primarily by homo- and heteromeric $P2X_2$ and $P2X_3$ receptors (Dunn *et al.* 2001). We have recently shown that currents mediated by homomeric $P2X_2$ receptors are attenuated under hypoxic conditions (Mason *et al.* 2003). This study examined the effect of hypoxia on homomeric $P2X_3$ and heteromeric $P2X_{2/3}$ receptors.

Whole-cell currents were recorded from $P2X_{2/3}$ or $P2X_3$ receptors stably expressed in HEK293 cells (Kawashima *et al.* 1998). Pipettes were filled with (mM): 10 NaCl, 117 KCl, 2 $MgSO_4$, 1 $CaCl_2$, 11 EGTA, 2 Na-ATP, 11 Hepes (pH 7.2), and cells were continuously perfused with 135 NaCl, 5 KCl, 1 EGTA, 5 Hepes and 10 glucose (pH 7.4). Cells were held at a potential of –70 mV and the cells perfused with either ATP or the $P2X_3$-selective agonist α,β-MeATP. Data are given as means ± S.E.M. and statistical analysis was performed using Student's paired or unpaired *t* test with $P < 0.05$ regarded as significant.

Perfusion of 0.5 μM ATP induced an inward current in cells expressing the $P2X_{2/3}$ receptor that showed little desensitization during repeated exposures under normoxic conditions (-70.0 ± 7.0 pA pF^{-1} to -66.4 ± 9.2 pA pF^{-1}, mean of first three and last three exposures respectively, $n = 5$). Exposure to a hypoxic ATP (0.5 μM) solution (P_{O_2} 25 mm Hg, EC solution bubbled with 100 % N_2) reduced the mean whole-cell current density from -100.8 ± 24.4 pA pF^{-1} to -62.8 ± 13.8 pA pF^{-1} ($P < 0.05$, $n = 5$). Similar results were obtained with hypoxic 10 μM α,β-MeATP (-32.7 ± 11.5 pA pF^{-1} to -19.5 ± 6.6 pA pF^{-1}, $P < 0.05$, $n = 6$). In contrast, in cells expressing the $P2X_3$ receptor, perfusion of ATP induced a rapidly desensitizing inward current. The magnitudes of the ATP-induced current densities were similar under normoxic (-18.7 ± 6.2 pA pF^{-1}, $n = 8$) and hypoxic (-24.4 ± 8.9 pA pF^{-1}, $n = 8$) conditions. Similar results were obtained with 10 μM α,β-MeATP (normoxic -18.7 ± 5.9 pA pF^{-1}, $n = 8$; hypoxic -19.7 ± 11.2 pA pF^{-1}, $n = 8$).

In summary, this is the first report indicating that hypoxia selectively modulates the response of P2X receptors to ATP and suggests that the presence of the $P2X_2$ receptor subunit in either homo- or heteromeric receptor channels is critical in mediating the response to hypoxia in the nervous system.

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Kawasima E *et al.* (1998). *Receptors Channels* **5**, 53–60.

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The authors would like to thank R.A. North (Institute of Molecular Physiology, University of Sheffield) for kindly providing us with the P2X stable cell lines. This work was funded by the Welcome Trust 065887/Z/01/Z.

PS C76

Electrically evoked and spontaneous mass GABA release in the island of Calleja complex does not require sodium channel activity

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The islands of Calleja (IC) are groupings of small granule cells (GCs) in the olfactory tubercles (OTs). The GCs have short local axons and are reportedly GABAergic (see Halliwell & Horne, 1998). They appear to envelop larger neurones that give rise to tubercle efferents. This study addresses the properties and activity of these larger cells associated with the IC, which I term satellite cells (SCs).

Coronal slices (200 μm) of OT from humanely killed rats aged 12–21 days were maintained conventionally *in vitro* at 25 °C. I made whole-cell recordings from SCs, visually identified with Nomarski optics. Eighty SCs were studied using pipettes including CsCl and 2–5 mM QX-314. Stimulating electrodes, which were broken patch electrodes (10 μm tip) filled with the bath solution and delivering cathodal current pulses, were placed amongst the GCs.

Stimulating in the IC at low current strength (< 20 μA, 200 μs) evoked synaptic activity that was predominantly blocked by 20 μM bicuculline (Bic) or gabazine (Gab, 0.5–10 μM). SCs loaded with CsCl and QX 314 displayed a high level of spontaneous input, which was blocked (> 90 %) by Bic or Gab. $GABA_A$ activity was isolated by adding 10 μM 6,7-dinitro-quinoxaline-2,3-dione (DNQX) and 10 μM D(–)-2-amino-5-phosphonopentanoic acid (D-AP5). All spontaneous activity was now blocked reversibly by Bic or Gab. Concomitantly, in 74 % of cells ($n = 47$) a significant outward current or hyperpolarization (according to voltage- or current-clamp conditions) developed following $GABA_A$ receptor blockade. Three kinds of spontaneous activity (at –60 mV) were observed in Cs^+-loaded SCs: (i) 38 % showed infrequent large currents (0.25–1 nA) or depolarizations (to ~0 mV) that lasted between 3 and 10 s – termed 'swoops'; (ii) 46 % had inward currents (150–500 pA) of up to 1 s duration (τ_{decay} >100 ms); (iii) all displayed shorter events (< 10–100 pA, $\tau_{decay} < 30$ ms) – 'minis'. All events were blocked by Bic or Gab. Swoops were not seen when 0.5–1 μM TTX was added to the bathing medium, but shorter events were resistant ($n = 12$). In medium containing only 50 μM Ca^{2+}, 6.25 mM Mg^{2+} and 1 mM Mn^{2+} as well as TTX, the intermediate spontaneous events were reduced in frequency but not totally abolished ($n = 4$). TTX, or an 83 % reduction of $[Na^+]_o$ by replacing Na^+ with choline, stopped IPSCs from being evoked by standard electrical stimuli; however, by increasing the amplitude and duration of the stimulus in the vicinity of the IC, Bic-sensitive IPSCs could once more be recruited in SCs ($n = 5$, each treatment).

From these results I conclude that (a) SCs receive a powerful local GABAergic input; (b) both desensitizing and non-desensitizing GABA receptors are present on SCs; (c) GABA release can be mobilized precipitately without the need for transmembrane Ca^{2+} flux; and (d) local GABA release sites can be recruited by purely electrotonic means to generate IPSCs in SCs. The most likely GABA source is a coupled network of IC granule cells (cf. Halliwell & Horne, 1998).

Halliwell JV & Horne AL (1998). *J Physiol* **506**, 175–194.

This work was supported by the Wellcome Trust.

All procedures accord with current UK legislation

PS C77

Cannabinoid CB1 receptor-mediated decreases in glutamatergic neurotransmission in the rat striatum involve a reduction in glutamate uptake

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Activation of cannabinoid CB1 receptors depresses excitatory neurotransmission in the striatum through a pre-synaptic mechanism believed to involve reduced glutamate release (Huang *et al.* 2001), although such a reduction has not been demonstrated directly. We have obtained evidence from biochemical experiments that CB1 receptor activation also reduces the uptake of glutamate in the striatum. Here we have investigated the role of glutamate reuptake in the effects of Δ^9-tetrahydrocannabinol (THC) on extracellular population spikes in rat striatal slices.

Coronal brain sections, 400 μm thick, were prepared from male Sprague-Dawley rats (7–9 weeks old), humanely killed by cervical dislocation. Hemislices containing the rostral striatum were used for electrophysiological recordings. Stimuli were delivered to the white matter overlying the striatum and extracellular responses recorded from the dorsolateral striatum. Effects of drugs on population spike amplitude (PSamp) are expressed as the mean ± S.E.M. percentage predrug values and analysed by one-way ANOVA followed by Tukey *post hoc* test.

A persistent depression in synaptic transmission was observed following treatment with 10 μM THC. Thus, PSamp was reduced to 65 ± 5 % predrug values 10–20 min after commencing THC washout ($n = 7$). THC did not reduce transmission when applied in the presence of AM251, a CB1 receptor antagonist (91 ± 3 % predrug; $n = 5$; $P < 0.05$ compared to THC alone). Inhibition of glutamate uptake by L-threo-hydroxyaspartate (THA; 500 μM) also reduced PSamp, but this effect was not prevented by AM251 (65 ± 4 % *vs.* 62 ± 10 %; $n = 8$; $P > 0.05$). In contrast, the actions of THA were reduced by pre-treatment with 10 μM LY341495, a broad-spectrum metabotropic glutamate receptor (mGluR) antagonist (82 ± 9 %; $n = 7$; $P < 0.05$ compared to THA alone). Agonists of group II and III, but not group I, mGluRs also produced synaptic depressions that could be reversed by application of LY341495.

When LY341495 was applied in the presence of THA, 10 μM THC no longer reduced PSamp in striatal slices (108 ± 18 %; $n = 5$; $P < 0.05$), suggesting the electrophysiological effects of this cannabinoid may involve reduced glutamate uptake. Similarly, the actions of 500 μM THA were attenuated when applied 20 min after THC application (83 ± 14 %; $n = 5$; $P < 0.05$). Furthermore, pre-treatment with 10 μM LY341495 prevented the THC-induced depression in PSamp (93 ± 4 % predrug; $n = 5$; $P < 0.05$).

These findings suggest that CB1 receptor-mediated depression of excitatory transmission in the striatum involves an indirect reduction in neurotransmitter release, by reducing glutamate re-uptake and consequently enhancing the activation of presynaptic inhibitory mGluRs.

Huang CC *et al.* (2001). *J Physiol* **532**, 731–748.

This work was funded by the BBSRC.

All procedures accord with current UK legislation

PS C78

Noradrenaline-mediated inhibition of TASK-1-like channels in neonatal rat facial motoneurones *in vitro*

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Members of the twin-pore domain potassium (K^+) channel family provide a molecular correlate for some neuronal 'leak' K^+ conductances. Recently mRNAs for two members of this family, the pH-sensitive TASK-1 and TASK-3 channels, have been demonstrated in rat facial motoneurones (FMs) (Rajan *et al.* 2000; Talley *et al.* 2000). We have used whole-cell patch-clamp recordings and neonatal rat brainstem slices, prepared from humanely killed rats, to investigate whether these channels underlie the 'leak' K^+ conductance inhibited by noradrenaline (NA) and 5-hydroxytryptamine (5-HT) in FMs (Larkman & Kelly, 1992, 1998).

Results were obtained from 25 different FMs voltage clamped at –60 mV in the presence of ZD 7288 (5 μM) to block the hyperpolarisation-activated current, I_h. The pH sensitivity of FM input conductance was determined by superfusing the slice with external solution titrated to a range of different pH. Lowering the external pH from 7.3 to 6 evoked an inward current of −63 ± 7 pA ($n = 9$, mean ± S.E.M.) associated with a conductance decrease. Raising the external pH to 8 evoked an outward current of +48 ± 5 pA ($n = 3$) and a conductance increase. Both inward and outward pH-sensitive currents (I_{pH}) had linear current–voltage (I–V) relationships and reversed close to the predicted K^+ equilibrium potential (E_K) (−93 ± 2 mV and −94 ± 4 mV for inward and outward I_{pH}, respectively). Averaged input conductance measurements ($n = 10$) at different external pH, expressed as a percentage of conductance at pH 7, could be described by a logistic function with a pK of 7.1 and a Hill coefficient of 1.4. The NA-sensitive current (I_{NA}) is also linear and reverses at the E_K (Larkman & Kelly, 1992). When external pH was lowered to 6.5, bath application of NA (10 μM) evoked an inward current of −34 ± 9 pA ($n = 5$). After increasing external pH to 7.7, NA evoked an inward current of −109 ± 19 pA in the same FMs ($n = 5$). External Ba^{2+} (200 μM to 2 mM) blocked both I_{NA} ($n = 4$) and I_{pH} ($n = 3$) at potentials negative to the reversal potential but were much less effective at potentials positive to the reversal level. External TEA chloride (30 mM) had no effect on I_{NA} or I_{pH}, but the addition of 4-AP (4 mM) blocked both I_{NA} and I_{pH} ($n = 3$). Zn^{2+} (100–500 μM) had no effect on I_{NA} or I_{pH} ($n = 3$).

In conclusion these biophysical and pharmacological data suggest a TASK-1-like channel mediates the 'leak' K^+ conductance and that inhibition of this conductance underlies NA-evoked depolarisation of rat FMs.

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Talley EM *et al.* (2000). *Neuron* **25**, 399–410.

This work was supported by the EU. E.M.P. holds a MRC studentship.

All procedures accord with current UK legislation

PS C79

Inhibition of nociceptive processing by μ-opioid receptor agonists in the rat superficial spinal dorsal horn *in vitro* is partly mediated by adenosine receptors

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Agonists at the μ-opioid receptor inhibit synaptic transmission throughout the brain and spinal cord. In the spinal dorsal horn, it has been suggested that μ-opioid agonists can also cause the release of adenosine, possibly by modulation of nucleoside transporters (Sweeney *et al*. 1993). However, the functional consequences of this release are unclear. We have used electrophysiological methods to assess whether μ-opioid-mediated release of adenosine impacts on synaptic processing in the superficial spinal dorsal horn *in vitro*.

Slices (300 μm) of lumbar spinal cord were obtained from 12- to 15-day-old Wistar rats irreversibly anaesthetised with urethane (I.P. 2 mg kg^{-1}). Whole cell patch clamp recordings were made from neurons within lamina II. Excitatory postsynaptic currents (EPSCs) were evoked in the presence of 30 μM bicuculline and 10 μM strychnine using a bipolar stimulating electrode placed onto the dorsal root entry zone. The μ-opioid receptor agonist [D-Ala^2,*N*-MePhe^4,Gly-ol^5]-enkephalin (DAMGO;1 μM), the adenosine A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 1 μM) and the opioid antagonist naloxone (10 μM) were bath applied. Data are expressed as the mean percentage inhibition of control ± S.E.M.

Application of DAMGO inhibited the EPSC by 32.9 ± 3.0 %. This inhibition was partially reversed to 13.1 ± 4.0 %, by co-application of DPCPX ($P < 0.01$, Student's paired *t* test, $n = 7$). Neither DPCPX nor naloxone had an effect on the EPSC amplitude when applied alone. In the presence of naloxone, DAMGO failed to produce an inhibition of the evoked EPSC (−2.9 ± 4.7 %, $n = 6$) suggesting that the effect of DAMGO was mediated specifically through opioid receptors rather than acting directly at adenosine receptors.

These data suggest therefore, that a component of opioid-mediated inhibition within substantia gelatinosa is via presynaptic A1 adenosine receptors. This is consistent with the proposal that μ-opioid receptor activation results in a release of adenosine (Sawynok *et al*. 1989) thereby further enhancing opioid-mediated inhibition of nociceptive transmission.

Sawynok J *et al.* (1989). *Trends Pharmacol Sci* **10**, 186–189.

Sweeney MI *et al.* (1993). *Brain Research* **614**, 301–307.

This work was supported by the MRC, UK.

All procedures accord with current UK legislation

PS C80

The effect of clonidine injection into the nucleus paragigantocellularis lateralis on nociception

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The paragigantocellularis lateralis (LPGi) is located in the rostroventrolateral medulla and involved in several functions such as cardiovascular regulation, sexual behavior, withdrawal syndrome, and especially nociception. α_2-Adrenoceptor agonists such as clonidine have been used for induction of analgesia in clinics. This drug affects the CNS, especially the nucleus locus coeruleus and the spinal cord. There is no information about the role of LPGi.

In this study the effects of clonidine administration into LPGi on the response to nociception (hot plate) were investigated. Twenty-eight male N-MRI adult male rats (220 ± 20 g) were used. Animals were divided into control and experimental groups. Experimental groups contained three subgroups: sham group that received artificial cerebrospinal fluid (ACSF) and two other subgroups that received 1μg/0.5μl and 2μg/0.5μl clonidine into the paragigantocellularis nucleus. Rats were anaesthetized with an injection (I.P.) of a mixture of ketamine hydrochloride (110 mg kg^{-1}) and Xylazine 2 % (5 mg kg^{-1}) and LPGi cannulated bilaterally in experimental animals under surgical procedure using the atlas of Paxinos & Watson (1998) (AP = 5.74, L = ± 1.6, DP = 12.12). After a recovery period, ACSF or clonidine (dissolved in ACSF) was injected into LPGi bilaterally and the response to pain was evaluated with the hot plate apparatus. All data were analysed using one-way ANOVA. At the end of the experiments the animals were humanely killed.

The results showed the following: (1) no difference was found between control and sham groups; (2) there was no significant difference between sham and 1μg/0.5μl clonidine groups; and (3) there was a significant difference between sham and 2μg/0.5μl clonidine groups ($P < 0.02$).

The results showed that clonidine injection to LPGi decreased response time to acute pain. It seems that α_2-adrenoceptors in LPGi modulate the pathway of pain and that the analgesic effect of clonidine is induced by α_2-adrenoceptors of the paragigantocellularis lateralis nucleus.

Paxinos & Watson (1998). *The Rat Brain in Stereotaxic Coordinates.* Academic Press, New York.

This project supported by Shahid Chamran University.

All procedures accord with current national and local guidelines

PS C82

Diverse fixed positional deformities and bone growth restraint are provoked by neuromuscular blockade-induced flaccid paralysis in embryonic chicks

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Pancuronium bromide (PB) is used in neonates and pregnant women to induce limp, flaccid paralysis in order to allow mechanical ventilation during intensive care. Such non-depolarising neuromuscular blocking drugs are administered to approximately 0.1 % of all human births in the UK.

In this study we examined the effects of PB administered via direct application to the chorioallantoic membrane on the skeletal development in White Leghorn chicken embryos. This involved daily administration (from day 10 gestation, for 1, 3, or 7days) of sterile PB in Tyrode solution (controls received Tyrode solution alone), weighing and examination of chicks killed humanely, before and after Alcian blue/Alizarin red staining of developing cartilage and bone skeletal elements. Direct video-imaging evidence of the efficacy of neuromuscular blockade has previously been described (Osborne *et al.* 2002).

We found that PB treatment produced skeletal deformities associated with significant reduction in longitudinal growth of all appendicular elements. The diverse range of fixed positional deformities, ranging from hyperflexion to hyperextension of individual joints such as the first inter-phalangeal joint, was associated with greater cartilage to bone ratios after prolonged periods of immobilsation (day 14 and 18), indicating a preferential reduction in osteogenesis. PB treatment also consistently increased incidence of knee joint flexion and tibiotarsal joint hyperextension. In addition to limb, spinal and craniofacial deformities, flaccid immobility appears to convert the normal geometric pattern of weight gain to a simple arithmetic accretion. Our preliminary results also indicate that a single PB dose, administered on day 10 produces sustained long-term immobility in 60 % of chicks at day 18. Intriguingly, similar positional deformities were seen in both these chicks, and those exhibiting recovery in normal motility (30 %) and weight gain.

This novel study highlights the potentially harmful effects of pharmacologically induced flaccid immobility on chick embryonic skeletal development. Whilst *in ovo* avian development clearly differs from human, our findings may have implications for the fetus and the premature and term neonate receiving such non-depolarising neuromuscular blocking drugs.

Osborne AC *et al.* (2002). *JMNI* **2**, 448–456.

All procedures accord with current UK legislation

PS C83

p-Chloroamphetamine-induced activity in the vas deferens and associated sympathetic nerve activity in the anaesthetised rat

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p-Chloroamphetamine (PCA)-induced penile erection and ejaculatory responses have previously been characterised in the urethane-anaesthetised rat (Yonezawa *et al.* 2000). The present study investigates activity in the vas deferens nerve (VDN) and intraluminal vas deferens pressure, and also tests the effects of the intravenous administration of PCA, an indirect 5-HT agonist.

Male Sprague-Dawley rats (300–325g) ($n = 4$) were anaesthetised (urethane 0.15–0.25 mg kg^{-1} I.V.). The VDN (a hypogastric branch) was identified in the abdominal cavity and isolated, and nerve activity recorded. A second group of rats ($n = 4$) were spinalised (T8/9, under urethane) and prepared as above. In two animals the ipsilateral vas deferens was isolated, and a 19G needle was inserted and connected to a pressure transducer and recorded. PCA was dissolved in saline (1.0 mg ml^{-1}) and given I.V. (1ml kg^{-1}). The effect of PCA on VDN activity and intraluminal vas deferens pressure was recorded. Animals were killed by an overdose of anaesthesic. Results are displayed as means ± S.E.M.

PCA evoked a sequence of responses, each consisting of synchronous bursts of activity ($n = 4$), beginning with a gradual increase (from 7.0 ± 1.1 Hz baseline) followed by several short bursts (3.8 ± 0.03 repeating at 0.36 ± 0.01 Hz) of escalating frequency (82.1 ± 16.0 Hz max). A single dose of PCA elicited 4.5 ± 0.3 responses lasting 19.7 ± 5.6 min. Intraluminal vas deferens pressure increased (18.6 ± 3.8 mmHg min to 24.1 ± 5.1 mmHg max) in bursts, as well as bulbocavernous muscles contractions, corresponding to bursts of nerve activity. Ejection of the seminal plug also occurred. One hour after the first dose, a subsequent PCA administration evoked further similar responses. Responses to PCA were observed unchanged in acutely spinalised animals. Removal of the efferent pathway by crushing the VDN central to the recording site abolished the PCA effects. Single shock stimulation of the VDN caused contraction of the vas deferens and an increase in intraluminal pressure.

This study shows that PCA-induced VDN activity closely correlates with ejaculatory reflexes and indicates that recording of VDN activity may be a valuable tool in the study of ejaculation. Furthermore we conclude that the rhythmic pattern of activity in the VDN associated with ejaculation represents the output of a spinal pattern generator like that suggested by Carro-Juárez *et al.* (2003). Importantly excitability of this pattern generator is enhanced by 5-HT-like receptor activation.

Carro-Juárez M *et al.* (2003). *Brain Res* **975**, 222–228.
Yonezawa *et al.* (2000). *Life Sci* **67**, 3031–3039.

This study was funded by BBSRC and Pfizer Ltd.

All procedures accord with current UK legislation