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DIFFERENCES IN STRIATAL SPINY NEURON ACTION POTENTIALS BETWEEN THE SPONTANEOUSLY HYPERTENSIVE AND WISTAR-KYOTO RAT STRAINS

T. L. PITCHER, J. R. WICKENS AND J. N. J. REYNOLDS*

Basal Ganglia Research Group, Department of Anatomy and Structural Biology, School of Medical Sciences, University of Otago, PO Box 913, Dunedin 9054, New Zealand

Abstract—The spontaneously hypertensive rat (SHR) and the Wistar-Kyoto (WKY) inbred rat strains display behavioral differences characterized by relative increases and decreases in levels of activity. Both strains have subsequently been utilized as animal models of hyperactive and hypoactive behavioral traits. The etiology of these behavioral characteristics is poorly understood, but may stem from alterations in the physiology of selected neural circuits or catecholamine systems. This study investigated the cellular properties of neurons from three genetically related strains: the SHR; WKY; and Wistar (WI). *In vivo* intracellular recordings were made under urethane anesthesia from spiny projection neurons in the striatum, a brain area involved in behavioral activation. Results obtained from 71 spiny projection neurons indicate that most cellular properties of these neurons were very similar across the three strains. However, the amplitude and half-duration of both spontaneously occurring and current-evoked action potentials were found to be significantly different between the SHR and WKY strains with neurons from the SHR firing action potentials of relatively greater amplitude and shorter duration. Action potential parameters measured from the WI rats were intermediate between the two other strains. These differences in action potentials between two behaviorally distinct strains may reflect altered functioning of particular membrane conductances.

Key words: SHR, WKY, striatum, urethane-anesthetized, intracellular recording.

The spontaneously hypertensive rat (SHR) was developed as an inbred strain to model aspects of human hypertension (Okamoto et al., 1972). During the selective breeding for high blood pressure, a number of behavioral characteristics were also fixed in the SHR genome. Some of these behavioral characteristics resemble those displayed in attention deficit hyperactivity disorder (ADHD) (Sagvolden et al., 1993, 1998). Interestingly, another genetically related strain, the Wistar-Kyoto (WKY) also displays abnormal behaviors and has subsequently been proposed as a model of depression (Pare, 1994; Malkesman et al., 2006). These strains are generally described as being hyperactive (SHR) and hypoactive (WKY), thus being at the opposite ends of the behavioral continuum.

The etiology of these behavioral anomalies is not known. However, some characteristics may be explained by alterations in dopamine functioning within the CNS (Viggiano et al., 2003). Alterations have been reported in both the SHR and WKY dopaminergic systems (Kujirai et al., 1990; Linthorst et al., 1991; Kirouac and Ganguly, 1993; Russell et al., 1995; Watanabe et al., 1997; Jiao et al., 2003; Mill et al., 2005). Whether a direct consequence of this altered dopamine function or not, differences in the physiology of brain areas involved in generating behavioral output could underlie the behavioral differences reported in these strains.

The basal ganglia is a neural circuit intimately involved in motor activity. The input nucleus of the basal ganglia, the striatum (caudate nucleus and putamen) receives extensive inputs from widespread areas of the cortex (McGeorge and Faull, 1989). Cortical information is processed in the striatum and output signals are sent via the spiny projection neurons to either the globus pallidus external segment (indirect pathway) or the globus pallidus internal segment (entopeduncular nucleus in rodents) or substantia nigra pars reticulata (direct pathway). Information is then relayed through the thalamus to the motor areas of the cortex. Information processing in the striatum is important for selection and initiation of motor sequences. Disruption of the striatal circuits, such as after the loss of dopaminergic inputs in Parkinson's disease or degeneration of striatal neurons in Huntington's disease, results in disorders dominated by motor abnormalities. Thus, variation in normal spiny projection neuron activity, mediated by alterations in cellular properties may also affect information flow through the basal ganglia and therefore, motor activity.

The present study utilized *in vivo* intracellular recording techniques to investigate the cellular properties of striatal spiny projection neurons in three rat strains: the SHR, a commonly used model of ADHD-like behaviors; the WKY, the normotensive genetic control for SHR and the standard albino Wistar (WI) strain. The purpose of the study was to investigate if there were, at the cellular level, physiological correlates to behavior.

EXPERIMENTAL PROCEDURES

Experimental subjects

Animals from the SHR, WKY and WI strains were obtained from the University of Otago Animal Facility (Dunedin, New Zealand).

*Corresponding author. Tel: +64-3-4795781; fax: +64-3-4797254.
E-mail address: john.reynolds@stonebow.otago.ac.nz (J. N. J. Reynolds).

Abbreviations: ADHD, attention deficit hyperactivity disorder; AHP, afterhyperpolarization; ANOVA, analysis of variance; SHR, spontaneously hypertensive rat; WI, Wistar; WKY, Wistar-Kyoto.

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These are maintained as inbred (SHR) and outbred (WKY and WI) colonies. The SHR and WKY are National Institutes of Health strains and were obtained from the Animal Resource Centre, Western Australia and have been maintained locally since late 2000. All surgical and experimental procedures were approved by the University of Otago's Animal Ethics Committee (97/01 and 93/04) as conforming to the New Zealand Animal Welfare Act (1999). All efforts were made to minimize the number of animals used and their suffering.

Animals were maintained in pairs or small groups on a reversed 12-h light/dark cycle with *ad libitum* access to food and water. The average age and weight of the animals on the day of experimentation was: SHR (n=23) 15.1±3.7 weeks and 320±33 g; WKY (n=21) 15.5±2.3 weeks and 308±24 g and WI (n=22) 10.8±2.1 weeks and 372±42 g. In general data were collected from one spiny projection neuron per animal, however in five animals (one SHR, two WKY and two WI) data were collected from two separate neurons per animal. SHR and WKY animals are known to weigh less than standard albino rats such as Sprague–Dawley at a similar age (Ferguson and Cada, 2003; Ferguson et al., 2003). Since efforts were made in the present study to record from rats in the weight range 300–400 g in accordance with our previous studies, there were significant differences in the ages of the SHR and WKY rats compared with the WI rats. Since all the animals used are considered in the adult age range, this difference is unlikely to have had a significant bearing on the results.

Stereotaxic surgery

On the day of experimentation animals were anesthetized with urethane (1.8–2.4 g/kg, i.p.; Sigma, Sydney, Australia) and fixed in the ear-bars of a stereotaxic frame. Core temperature was maintained at 36.5±0.5 °C with a homeothermic blanket and rectal probe. Local anesthetic (bupivacaine 0.5%; AstraZeneca, Auckland, New Zealand) was injected into the scalp and the skull exposed by a midsagittal incision. Burr holes were drilled in the skull for placement of an anchoring screw and an electrode over the right parietal cortex, contralateral to the recording site, in order to record the electrocorticogram. The electrocorticogram waveform was used as an indication of depth of anesthesia and supplementary anesthesia (ketamine 10 mg/kg and xylazine 2 mg/kg or urethane 0.6–0.7 g/kg; Parnell Laboratories New Zealand Ltd., Auckland, New Zealand) was given accordingly. A craniotomy was performed over the left striatum (AP; +8.5 to +12.5 mm, ML; +1.0 to +4.5 mm). Dental acrylic was used to secure the electrocorticogram electrode to the skull and to form a 'well' around the craniotomy. During recording the 'well' was filled with paraffin wax to maintain stability.

Cellular properties were compared in neurons recorded under urethane only and urethane with ketamine and xylazine supplement. There were no differences in cellular properties (data not shown); hence animals recorded under both anesthetic regimens are pooled.

Intracellular recording

Intracellular records from striatal spiny projection neurons were made with 1 M potassium acetate-filled microelectrodes (70–120 MΩ resistance) pulled from 3.0 mm diameter glass capillaries (Harvard Apparatus, Edenbridge, UK). Neuron impalement was aided by passing current pulses through the microelectrode. Neurons included in the study displayed a down-state membrane potential of at least −60 mV and had action potentials with amplitudes of greater than 50 mV, which overshot 0 mV. Spontaneous activity from each neuron was recorded (Fig. 1A) and neuronal responses to current pulses (Fig. 1 C) were collected at the onset of a down-state with the use of a threshold discriminator (Reynolds and Wickens, 2003). These responses were collected for measurement of the current/voltage relationships and basic action potential properties.

Measurement of cellular properties

The average membrane potential in both the down- and up-states of spiny projection neurons was estimated by fitting a weighted sum of three Gaussian curves to an all-points histogram constructed from a 30-s period of spontaneous activity (Fig. 1B). This method is similar to that described previously (Reynolds and Wickens, 2000). Input resistance of each neuron was estimated from the slope of a regression line fitted to subthreshold depolarizing current pulses (Fig. 1C and D).

Single action potentials were evoked by a 100 ms depolarizing current pulse triggered in the down-state (digitizing rate of 20 kHz). The intensity of the rheobasic current was set to ensure the firing of a single action potential, delayed by at least 50 ms. Action potential values were measured from all traces containing a single action potential. Parameters were measured as illustrated in Fig. 1E. Briefly, action potential threshold was defined as the point on the voltage trajectory when the rate of depolarization reached 8 V/s, using a moving window of 10 points to interpolate the slope. Action potential amplitude was defined as the distance from threshold to positive peak and action potential half-duration was measured at half-amplitude level. Action potential firing latency was measured from current pulse onset to the time of action potential threshold. Afterhyperpolarization (AHP) amplitude was measured as the distance from the threshold to the maximum negative peak occurring after the action potential. Parameter values for each neuron were an average taken from at least five single action potentials.

Average action potential traces were constructed by normalizing (to the time of action potential threshold) and averaging all traces containing a single action potential for each neuron. Group averages were then constructed from these individual neuron averages. Action potential depolarizing and repolarizing slope measurements were made on the average action potential traces from each neuron (Fig. 1F). All offline analyses were performed in Axograph 4.8 (Axon Instruments, Foster City, CA, USA).

Histology

Some neurons were passively filled during the recording period with Biocytin (4%; Sigma). Paraformaldehyde perfusion-fixed brains were sectioned on a vibratome (50 μm) and processed using standard histological techniques (Horikawa and Armstrong, 1988). Filled neurons were viewed under the light microscope.

Statistical analysis

Cellular property measurements of spiny projection neurons were compared using a one-way analysis of variance (ANOVA), with Tukey's B post hoc analysis. Significance was set at $P<0.05$.

RESULTS

Recordings were obtained from 71 neurons identified as spiny projection neurons on the basis of their electrophysiological properties. These properties are characteristic of spiny projection neurons reported previously by our laboratory (Reynolds and Wickens, 2004) and by others in the literature (Wilson and Groves, 1981; Mahon et al., 2001). Examples of spiny projection neuron morphology from each strain are shown in Fig. 2. There did not appear to be any obvious differences in morphology between neurons from the three strains, however, no quantitative analysis was carried out. Measurements of cellular properties from each of