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Intracellular pH rises and astrocytes swell after portacaval anastomosis in rats

MARGARET S. SWAIN, ANDRES T. BLEI, ROGER F. BUTTERWORTH, and RICHARD P. KRAIG

Departments of Neurology and Pharmacological and Physiological Sciences, The University of Chicago, Chicago, Illinois 60637

Abstract

The basis for astrocytic swelling after the early period after portacaval anastomosis (PCA) is poorly defined. In other eukaryotic cells intracellular pH (pH_i) and volume are determined, in part, by the same general mechanisms, yet how astrocytic pH_i varies with enlargement of these cells after PCA is unknown. Therefore, direct measurements of pH_i in astrocytes were made and compared with pericapillary astrocytic area as determined from electron micrographs in rats 5–8 days after PCA. Astrocytic area ($n = 14$ measurements for each group) was found to be significantly ($P < 0.0009$) greater in PCA animals ($n = 3$) than in sham-operated control animals. ($n = 3$). Double-barrel pH-sensitive microelectrodes were used to measure pH_i in neocortical cells defined by electrophysiological criteria to be astrocytic. Astrocytes ($n = 25$) from PCA animals ($n = 5$) had a resting membrane potential of 72 ± 5 mV (mean $\pm$ SD) and an pH_i of 7.11 ± 0.11 while comparable cells ($n = 12$) from sham-operated controls ($n = 2$) had a membrane potential of 81 ± 6 mV and an pH_i of 7.00 ± 0.10 . Astrocytes from PCA animals were significantly more depolarized ($P < 0.001$) and alkaline ($P < 0.009$), at a time when they were also significantly larger than those from sham-operated controls. Astrocytes are known to become more alkaline when they are activated by brief depolarizing stimuli. However, this is the first demonstration that such an interrelationship can also exist for steady-state conditions of these cells. Furthermore, these results provide direct support for the suggestion that astrocytic pH_i can be modulated in parallel with the volume of these cells.

Keywords

liver; hyperammonemia; hepatic encephalopathy; brain edema; ammonia

Chronically impaired liver function induces a characteristic change in astrocyte morphology that can be experimentally generated in the rat after construction of a portacaval anastomosis (PCA). Diversion of the portal venous blood from the liver into the systemic circulation results in accumulation of brain ammonia, which, in the early postshunt period, is associated with selective swelling of astrocytes (26,33); astrocytic changes characterize portal-systemic encephalopathy as well as congenital hyperammonemic syndromes in humans (9).

Address for reprint requests: R. P. Kraig, Dept. of Neurology, The University of Chicago, 5841 South Maryland Ave., Chicago, IL 60637. Current address for M. Swain and R. F. Butterworth: Andre-Viallet Clinical Research Center, Hospital St. Luc and University of Montreal, Montreal, Quebec H2X354, Canada.

Current address for A. T. Blei: Lakeside Veterans Administration Medical Center, Department of Medicine, 333 East Huron Street, Chicago, IL 60611.

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Cellular mechanisms that account for the unique enlargement of astrocytes after PCA are controversial. Astrocytic swelling in hyperammonemia could result from ammonia-induced alterations in cerebral energy metabolism (13). However, astrocytes are intimately involved in brain ammonia detoxification and are implicated in the metabolism of the neurotransmitter amino acids, aspartate and glutamate (3). After PCA, brain interstitial glutamate concentrations rise as a result of decreased reuptake into perineuronal astrocytes (10). Thus the increase in astrocytic size seen after PCA may be related to enhanced metabolic activity associated with ammonia detoxification (13,33). Alternatively, in many eukaryotic cells intracellular pH (pH_i) and volume are largely determined by the same general mechanisms and thus could simultaneously change (17). Here we provide the first direct evidence that after PCA the pH_i of astrocytes is significantly greater than normal at a time when the pericapillary processes of these cells are significantly increased in size.

METHODS

Animal preparation and recording

Wistar rats ($n = 7$; 350–400 g) were anesthetized with halothane (5% induction, 3% during surgical procedures, and 1% during electrophysiological recordings) and an end-to-side PCA was created ($n = 5$) (27). Other animals ($n = 2$) underwent a sham operation that consisted of a laparotomy and clamping of the portal vein with partial clamping of the vena cava for 15 min (as done in the PCA group). Animals were allowed to recover for 5–8 days in individual cages with a 12:12 h light-dark cycle at 22°C with free access to food and water. No evidence of shunt occlusion was seen at the time of death.

For electrophysiological recordings, PCA animals were prepared as previously described (11,12). Briefly, animals were reanesthetized with halothane, a tracheostomy was made, and a tail artery was cannulated. A craniotomy was made over frontal cortex (3 mm anterior to bregma and 2 mm lateral to the sagittal suture), and animals were mounted in a standard stereotaxic apparatus. Brain pulsations were minimized by draining cerebrospinal fluid via a cisternal incision, by bilateral pneumothoraxes, and by placement of a superfusion cup-pressure foot over the exposed pial surface.

Throughout the recording periods the exposed frontal cortex was isolated with agar, while the superfusion cup was bathed with warmed (35–37°C) Ringer solution containing (in mM) 108 NaCl, 3 KCl, 26 NaHCO_3 , 1.5 CaCl_2 , 1.4 MgCl_2 , 5 glucose, 8 sucrose, and 10 sodium gluconate, which when aerated with 5% CO_2 –95% O_2 had a pH of 7.3–7.4 (at 25°C) (modified from Ref. 6). Anesthetized animals were immobilized with *d*-tubocurarine chloride (1.5 mg/kg) and mechanically ventilated with a 30% O_2 –70% N_2 gas mixture. Body temperature was maintained at $37 \pm 0.5^\circ\text{C}$. Arterial blood variables (pH and arterial CO_2 and O_2 tensions) were normalized and monitored with a Corning 168 blood gas analyzer (Ciba Corning Diagnostics, Medfield, MA). The systolic arterial blood pressure was recorded continuously, and blood glucose was periodically measured with a Glucometer (Miles Laboratories, Naperville, IL). Double-barrel pH-sensitive ion-selective micropipettes (ISM), based on the tri-dodecylamine ionophore (2), were constructed as previously described (22) except that ISMs were silanized with a 1:3 mixture of dimethyltrimethylsilylamine (Fluka; Basel, Switzerland) in xylene. The inner reference barrel contained 0.5 M KCl and had an impedance of 175–443 M Ω . ISMs were calibrated in phosphate buffers (6.0–7.4 pH) before and after recordings, and if an ISM slope changed by >5 mV/pH after recording, the data were discarded. Initial ISM slope response (mV/pH) was 55 ± 3 mV/pH ($n = 11$). Brain ISM recordings were referenced to the pH of Ringer superfusate that was periodically monitored with a semimicro-pH electrode (model 410; Microelectrodes, Londonderry, NH). ISMs were connected to an A-I Axoprobe amplifier system (Axon Instruments; Burlingame, CA). A 1 M KCl, 3% agar bridge placed on a temporalis muscle served as the distant indifferent electrode. Reference barrel potentials were

electronically subtracted from ion-barrel potentials to yield a pure pH signal. Data were filtered at 2 Hz and visualized on a strip chart recorder. Fast (ms time scale) signals were visualized on an oscilloscope (model 2090; Nicolet Instrument, Madison, WI). All electrophysiological signals were stored with a videocassette recorder (model DR-484; Neurodata Instruments, New York, NY). Cells were identified as neurons or glia by classical electrophysiological criteria (32). Spreading depression was induced by stimulating the cortical surface in a train (100 Hz, 840 ms duration) repeated at 1 Hz for 1–2 s, via two 0.5-mm-diameter insulated flattened stainless steel rods positioned 3.5 mm apart on either side of the super-fusion cup (11,12). Cell membrane impedance and time constants were determined by the bridge imbalance technique.

Electron microscopy

A second group of experimental animals ($n = 6$) underwent creation of a PCA ($n = 3$). Sham-operated animals ($n = 3$) were prepared as described above. Animals were allowed to survive for 6 days and were then reanesthetized and killed by perfusion fixation (2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer; 7.4 pH). Animals were decapitated, and the heads were stored in fixative for 2–3 h. The brains were then removed, cut into 1-mm-thick coronal sections, and stored in 0.1 M phosphate buffer (7.4 pH). Tissue sections were subsequently washed with phosphate buffer, postfixed with 1% osmic acid, dehydrated through an ethyl alcohol series, cleared in propylene oxide, and embedded in Epon. The embedded tissue was sectioned on an MT2B ultramicrotome, stained with uranyl acetate and lead nitrate, and examined in a Philips CM 10 electron microscope (Inahaven, The Netherlands) using a 200- μm condenser aperture and a 40- μm objective aperture. Micrographs were obtained at an optical magnification of 5,000–10,000 and photographically enlarged to final magnifications.

Relative pericapillary astrocytic area was measured with a computer-based image analysis system (Bioquant-IV; R & M Biometrics, Nashville, TN) and normalized by dividing these values by similarly measured adjacent capillary area. Astrocyte-to-capillary ratios were compared between sham-operated and experimental animals using a two-independent-variable t test calculated via Systat 4.2 (Systat, Evanston, IL). Two-sided P values are reported. Other variables were similarly compared, and all variables were expressed as means $\pm$ SD and range.

RESULTS

Pericapillary astrocytic area was $7 \pm 4 \mu\text{m}^2$ in sham-operated control animals and $27 \pm 26 \mu\text{m}^2$ in PCA animals, while adjacent capillary area was $36 \pm 36 \mu\text{m}^2$ and $34 \pm 21 \mu\text{m}^2$, respectively, in these two groups. The ratio ($n = 14$) of pericapillary astrocytic area compared with adjacent capillary area was significantly greater ($P < 0.0009$, $t = 4.683$) in PCA animals ($n = 3$) than it ($n = 14$) was in sham-operated control animals ($n = 3$). An example of this difference in pericapillary astrocytic area is shown in Fig. 1. Astrocytic swelling was also observed in processes not adjacent to capillaries (data not shown). However, these latter processes were not systematically measured and so were not quantitatively evaluated. All electron micrographs were made from tissue samples that varied in depth throughout frontal neocortex so they would be directly comparable to electrode recordings from astrocytes that were obtained from a similar range in depth in frontal neocortex.

Arterial blood physiological variables are shown in Table 1 and were typical for anesthetized and artificially ventilated rats (11,12,28). There was no significant difference ($P > 0.744$, $t = 0.359$) between the arterial pH of PCA and sham-operated control animals. On the other hand, brain interstitial pH was significantly ($P < 0.045$, $t = 2.258$) more alkaline in PCA animals than in sham-operated controls (Table 2). The pH of the interstitial space in PCA animals was 7.36 ± 0.07 ($n = 15$ measurements in 2 animals; range: 7.21–7.49) while that of sham-operated controls was 7.28 ± 0.08 ($n = 8$ measurements in 2 animals; range: 7.16–7.38). The pH of interstitial space was measured at various neocortical depths in both groups.

Glial cells were identified upon penetration with ISMs by the classical electrophysiological criteria of a high membrane potential, low membrane impedance and time constant, and absence of any injury or evoked electro-genic activity (32). Takato and Goldring (32) noted that when micropipettes are passed through neocortex and similar electrophysiological observations are seen, recordings are invariably from protoplasmic astrocytes. Indeed, horseradish peroxidase injections have been used in previous studies (11) to show that when pH-ISMs penetrate neocortical glial cells, recordings are invariably made from protoplasmic astrocytes. Thus we presume the glial cells penetrated here were also protoplasmic astrocytes. All cellular penetrations classified as astrocytes had a high membrane potential (≥ 65 mV), a membrane impedance of <5 M Ω , and showed no injury or evoked electrogenic activity. In animals that received a PCA ($n = 5$), recordings from 25 cells indicated a resting membrane potential of 72 ± 5 mV (range: 65–83 mV) and intracellular pH of 7.11 ± 0.11 {range: 6.85–7.34}. Sham-operated control animals ($n = 2$) had astrocytes ($n = 12$) with a membrane potential of 81 ± 6 mV (range: 70–93 mV) and a pH_i of 7.00 ± 0.10 (range: 6.83–7.12). These results are significantly different with astrocytes being more depolarized ($P < 0.001$, $t = 3.962$) and more alkaline ($P < 0.009$, $t = 2.819$) in PCA animals than in sham-operated controls (Table 2).

To further illustrate this rise in astrocytic pH_i after PCA the above data were compared with those derived from normal astrocytes (12) under analogous recording conditions. In 53 astrocytes, defined as described above, membrane potential was 73 ± 6 mV (range: 66–88 mV) and pH_i was 7.04 ± 0.13 . Astrocytic pH_i of sham-operated controls was not significantly different ($P > 0.230$, $t = 1.237$) from that seen in normal astrocytes (12); thus these groups could be pooled into one population. Such grouping did not influence the fact that the pH_i of astrocytes from PCA animals was still significantly ($P < 0.015$, $t = 2.517$) more alkaline than that of normals plus sham-operated controls (7.04 ± 0.12 , $n = 65$). On the other hand, astrocytic membrane potential was significantly different ($P < 0.003$, $t = 3.503$) between normal animals (12) and the sham-operated controls examined in this study. Furthermore, if the membrane potential results of sham-operated controls and normal animals were grouped together, their average (75 ± 6 mV; $n = 65$) was no longer significantly different ($P > 0.062$, $t = 1.893$) from that of astrocytes from PCA animals (Table 2). These latter significant and nonsignificant differences in astrocytic membrane potential may reflect an improved recording ability (i.e., higher membrane potential) in our current study compared with the original recordings made in this laboratory (12).

In contrast to differences in astrocytic membrane potential and pH_i under steady-state conditions, PCA does not seem to influence the dynamic behavior of astrocytic membrane potential and pH_i . This conclusion stems from the observed behavior of these parameters during evoked neuronal stimulation and spreading depression (Fig. 2). Spreading depression is a propagating and transient loss of membrane potential and electrophysiological activity in brain cells of susceptible neural tissues (7). Spreading depression causes a large depolarization and alkalization of astrocytes where pH_i rises from its resting level of 7.0–7.1 to 7.4–7.6 (11, 12). Only after these cells repolarize does their pH_i swing transiently more acid than baseline before quickly settling back to its resting level. Evoked neuronal stimulation causes a smaller but similar pattern of potential and pH_i change (11,12). Spreading depression and evoked neuronal activity were elicited during five astrocytic recordings from PCA animals. The peak alkaline transient seen during spreading depression ranged from 7.21 to 7.49. An example of these astrocytic membrane potential and pH_i changes during spreading depression and evoked neuronal activity in a PCA animal is shown in Fig. 2.

DISCUSSION

This study, employing pH-sensitive ISMs, demonstrated that the pH_i of astrocytes from animals that had undergone PCA was significantly more alkaline and depolarized than those from sham-

operated controls. Furthermore, astrocytic endfoot processes in PCA animals were significantly larger than the comparable cellular appendages in sham-operated animals. Taken together, these results represent direct evidence that astrocytes *in vivo* can have a pH_i greater than normal at a time when their size is also significantly increased.

Brain pH_i after PCA has been measured previously. Using nuclear magnetic resonance (NMR) spectroscopy, average brain pH_i was not found to be significantly different in rats after PCA compared with nonshunted weight-matched rats (14). The astrocytic pH_i measurements reported in this study were completed 5–8 days after PCA, while those by Fitzpatrick and co-workers (14) were done 4 wk after PCA. Perhaps astrocytic pH_i returns to normal 4 wk after PCA. Alternatively, NMR spectroscopy may be too insensitive to detect an elevation of astrocytic pH_i by 0.11. Neuronal pH_i was not systematically measured in this study because recordings from these cells are technically much more difficult to attain. If neuronal pH_i does not rise along with astrocytic pH_i after PCA, NMR spectroscopy may be unable to detect the astrocytic change because the technique cannot resolve cellular differences on the order of 0.05–0.10 in pH_i within tissues consisting of heterogeneous (i.e., neuronal and glial) cell types (1). Furthermore, astrocytic swelling after PCA is confined to neocortical, and not white matter, astrocytes (26,33). If a rise in astrocytic pH_i after PCA has a similar distribution, the volume of involved cells may be too small for detection, since NMR spectroscopy measures the average pH_i of whole brain. Finally, if neuronal pH_i does rise by a similar degree to that seen in astrocytes NMR spectroscopy may be unable to detect it because the change approaches the limit of resolution of the technique (1).

A precedent exists for the observation that pH_i can rise as eukaryotic cells enlarge. In the presence of bicarbonate, cultured rat hepatocytes exhibit a rise in pH_i of 0.10–0.40 as they swell from a previously shrunken state (15). Similarly, cultured rodent osteoclasts undergo a similar rise in pH_i when they swell from exposure to hypotonic media (16). In both of these instances increased activity of a plasma membrane-based sodium-hydrogen antiport system is believed to be the basis for the observed rise in pH_i . The cellular basis of increased astrocytic pH_i after PCA is likely to be more complex, however, if it and the pH of the interstitial space are determined by the same mechanisms. This conclusion stems from the fact that although increased sodium-hydrogen antiport activity would raise pH_i it would lower interstitial pH. Yet this study demonstrated that PCA causes a significant increase in both astrocytic and interstitial pH. A third example of cellular pH_i and volume behavior analogous to that reported in this study comes from preliminary data derived from mammalian brain showing that astrocytes can undergo a rise in pH_i as the cells enlarge from stimuli other than PCA (23,24).

Indeed, astrocytes can swell as their pH_i shifts in both the alkaline and acid direction. For example, astrocytic pH_i rises from an average baseline of 7.04 by 0.11–0.78 during spreading depression (11,12) at a time when the interstitial space shrinks (29), perhaps because of potassium-induced swelling of these cells (4,23). In addition astrocytes within mammalian cerebral cortex also enlarge (19; see Ref. 21 for review) during global ischemia when their pH_i rises by 0.08 (23). Furthermore, shortly after reperfusion from global ischemia, astrocytes are most swollen when their pH_i has shifted by 0.24–0.44 to a most acidic level from baseline (24). The molecular bases for alterations in astrocytic pH_i and volume are unknown. In hepatocytes (15) and osteoclasts (16) a rise in pH_i and volume is, at least in part, due to plasma membrane-based sodium-hydrogen antiport. Such counter-transport activity may also apply to the pH_i /volume behavior of astrocytes, since this antiport mechanism is known to be present in these cells (5). On the other hand, since an increase in astrocytic pH_i is accompanied by a decrease in cell polarization, attention should also be given to volume regulatory mechanisms that are associated with depolarization.

Depolarization induced by neuronal activity (12), spreading depression (11,12), and even global ischemia (under normoglycemic conditions) (23) is associated with a rise in astrocytic pH_i . Each of these examples can be considered more dynamic than the “steady-state-like” conditions of PCA employed in this study. Nonetheless, PCA also reveals the same correlation of membrane potential and pH_i of astrocytes. Furthermore, since cultured astrocytes (5), oligodendrocytes (20), and renal epithelial cells (25) show a similar pH_i change to depolarizing stimuli, it may be of general significance. Depolarization-coupled (i.e., potassium-induced) swelling of astrocytes can result from a net accumulation of sodium and chloride via the activity of various membrane transport systems (21). These same and other pH-related transport systems could account for both swelling and pH_i rise seen in these cells after PCA.

Alternatively, alterations in cellular metabolic processes induced by hyperammonemia from PCA may result in an increase of intracellular osmolality and so prompt cell swelling (13,21). Brain ammonia content rises by <0.5 mM 7 days after PCA (18). If astrocytic physicochemical buffer capacity approximates that of whole brain homogenates [i.e., 35 mM/pH (30)], this increase in ammonia is too low to account for the 0.11 rise in pH_i seen in astrocytes. On the other hand, glutamine, which has an ionization equilibrium constant near that of ammonia, rises to ~ 15 mM/kg neocortex after PCA (9) and thus could account for the rise in astrocytic pH_i seen. Indeed, accumulation of glutamine has been proposed as a mediator of water increase in the brain (and astrocytic swelling?) of hyper-ammonemic rats (32).

Intracellular acid-base status has long been considered to be capable of influencing cellular metabolism. Recently, Busa and Nucitelli (8) suggested that pH_i could modulate cellular activity by creating an environment conducive to specific vital activities. Their speculation is based on data that show that a rise in pH_i correlates to an increase in metabolic processes, while a decrease in pH_i is associated with dormancy. For example, a rise in pH_i on the order of 0.05–0.40 is associated with fertilization of frog (*Xenopus*) eggs and sea urchin (*Lytechinus pictus*) eggs, initiation of protein synthesis in a cell-free system derived from sea urchin eggs, insulin stimulation of glycolysis in frog muscle, and the initiation of development in cultured cells that are exposed to growth factors (for review see Ref. 8). The rise in astrocytic pH_i seen after PCA may be a necessary concomitant of cellular volume regulatory processes and, perhaps, is also a purposeful alteration in astrocytic acid-base status destined to initiate vital activities pertinent to astrocytic reaction to hyperammonemia from PCA.

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FIG. 1.

Astrocytic swelling after a portacaval shunt (PCA). Electron micrograph at left shows capillary and surrounding neuropil from sham-operated control animal. Animal was allowed to survive 6 days after sham surgical procedure to abdomen. Astrocytic endfoot processes encircling capillary are thin and of typical density for these cells (indicated in lower right quadrant by white arrowheads). Comparable capillary with surrounding swollen astrocytic processes is shown in micrograph at right. The latter image was made from an animal allowed to survive for 6 days after creation of a PCA. Three sham and three experimental animals were prepared and allowed to survive for 6 days before their brains were prepared for electron micrographic examination of pericapillary astrocytic swelling. Astrocytic areas were measured with a computer-based image analysis system and normalized by dividing by comparably measured adjacent capillary area. Micrographs ($n = 14$) from PCA animals showed a significant ($P < 0.0009$; $t = 4.682$) increase in pericapillary astrocytic area compared with control images ($n = 14$). Calibration bar in both micrographs is equal to $2 \mu\text{m}$

FIG. 2.

Astrocytic pH_i after portacaval shunting. *Top*: upper record shows pH_i and lower record shows membrane potential from a cell defined as glial by classical electrophysiological criteria and presumed to be astrocytic. Spreading depression was elicited by 100-Hz surface electrical stimulation for 2 s (arrow) and was heralded by the subsequent large transient depolarization of ~30 s in duration. The latter potential change is correlated with a concomitant rise in pH_i, a change seen in normal astrocytes during spreading depression. In this example, astrocytic pH_i was 6.97 and rose to 7.33 during spreading depression before reaching an acid-going peak of 6.95 when the cell repolarized. Similarly, during 20-Hz surface electrical stimulation (horizontal bar) membrane potential transiently declined and pH_i rose. *Bottom*: histograms

show frequency and distribution of pH_i recordings in PCA animals (*left*) and sham animals (*right*).

TABLE 1

Arterial blood physiological variables

	Sham-Operated Control Animals (<i>n</i> = 2)	Portocaval-Shunted Animals (<i>n</i> = 5)
pH	7.41 ± 0.04	7.42 ± 0.03
PaCO ₂ , Torr	32 ± 3	34 ± 3
PaO ₂ , Torr	129 ± 19	130 ± 7
SBP, Torr	114 ± 8	101 ± 6
Hct, %	42 ± 0	40 ± 3
Glucose, mM	6.9 ± 0.4	6.5 ± 0.7
Temperature, °C	37.0 ± 0.4	37.0 ± 0.2

Values are means ± SD. PaCO₂, arterial CO₂ tension; PaO₂, arterial O₂ tension; Hct, hematocrit; SBP, systolic blood pressure.

TABLE 2

Astrocytic and interstitial pH

	Astrocytes	Interstitial Space
<i>Portacaval-shunted animals (n=5)</i>		
pH		
Means±SE	7.11±0.11	7.36±0.07
Range	6.85–7.34	7.21–7.49
No. of measurements	25	15
<i>P</i>	<0.009 [*] ; <0.015 [†]	<0.045 [*]
Membrane potential		
Means±SE, mV	72±5	
Range, mV	65–83	
No. of measurements	25	
<i>P</i>	<0.001 [*] ; >0.062 [†]	
<i>Sham-operated animals (n = 2)</i>		
pH		
Means±SE	7.00±0.10	7.28±0.08
Range	6.83–7.12	7.16–7.38
No. of observations	12	8
<i>P</i>	>0.230 [†]	
Membrane potential		
Means±SE, mV	81±6	
Range, mV	70–93	
No. of observations	12	
<i>P</i>	<0.003 [‡]	
<i>Normal animals[§] (n = 30)</i>		
pH		
Mean±SE	7.04±0.13	
Range	6.80–7.26	
No. of observations	53	
Membrane potential		
Mean±SE (mV)	73±6	
Range	66–88	
No. of observations	53	

* PCA animals vs. sham-operated controls;

[†] PCA animals vs. the sum of sham-operated controls and normal animals from Ref. 12;

[‡] Sham-operated control animals vs. normal animals from Ref. 12;

[§] Values from Ref. 12.