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Regulation of Na^+/K^+-ATPase and Plasma Membrane Calcium ATPase in Brain-Gut Axis during Restraint Stress in Ageing Male Mice

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Abstract

Ageing is believed to be a continuous process that begins at conception and proceeds until death. Little is known about the response of mice to ageing and restraint stress. Therefore, in this study, BALB/c mice of different age groups (1, 2, 4 and 6 months) were subjected to restraint stress of 30 min for two consecutive days. Ion transporters being the ion homeostasis regulators of the cell, we explored the response of Na^+/K^+-ATPase (NKA) and Plasma Membrane Calcium ATPase (PMCA) to restraint stress, an acute stressor. We examined the activity pattern of these ATPases in mice gut (fundus and pyloric regions of the stomach, the duodenum and the jejunum) and brain (cortex, hippocampus and cerebellum) in the stressed condition. The pattern of NKA and PMCA activities showed significant shift in stressed mice that corresponds with increasing age. This differential pattern of ion transporter response in the varied regions of the brain and gut present physiological evidence for a spatio-temporal modification of ion-transporter activity during ageing and restraint stress. Overall, the present data point to a vital role of brain-gut axis in the regulation of ion homeostasis in male mice.

Keywords: Ageing, Brain-gut Axis, Na^+/K^+ATPase, Mice, PMCA, Restraint Stress

1. Introduction

The ageing process begins even before an organism is born[1]. Ageing is a multi-factorial process, which comprises of both intrinsic and extrinsic factors. It is a continuous process that initiates at conception and continues until the death of an individual[2]. Mammalian lifespan comprises of early postnatal, pre-pubescent, adolescent, adulthood and old age. Early postnatal stage in mice starts from birth to weaning period, which is the 21st day of the postnatal period. The pre-pubescent stage starts at weaning and is characterised by a rise in growth hormones, which peaks at Postnatal Day (PND) 28-30[3]. Mice in the pre-pubescent period may be well thought out as juveniles. Adolescence is commonly considered to be between childhood and adulthood that comes on the second month of the postnatal period[4]. During this phase a range of hormonal and behavioural alterations in neurobiological structures occur. Moreover, adult reference group for studies on mice is usually between three to six months[5]. It is considered that two to three-month period is classically kept as a switching period between adolescence and adulthood, where the speed of development is steep[6]. Stress and ageing mimic each

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other but sometimes interact paradoxically with stress being able to either relieve or intensify the ageing process and, similarly ageing can cause stress to have a stronger or weaker impact, and these interactive effects remain a mystery[7]. Restraint or immobilisation technique is the most broadly used models to explore the physiological and behavioral effects of stress exposure in laboratory animals, particularly in rodents[8].

The study focused on the period when the mice are more homeostatically stable, yet the physiological and molecular changes associated with stress are explored. Therefore, in this study, mice aged one month (juveniles), two months (adolescents), four months (young adults) and six months (mature adults) were considered. Mice in early postnatal period and senescent period were avoided because the early postnatal period is the time when the mice grow and stabilise physiologically whereas, during ageing, a complete destabilisation of homeostasis takes place. Additionally, the available literature on ageing primarily focuses on the early postnatal period and senescence.

The ATPases are integral membrane proteins that are accountable for the transport of ions through the cell membrane. Hence, any alteration in their activities will replicate modifications in the functional status of the plasma membrane. The effect of acute stress on the functions of ion transporters in the gut and brain was studied to clarify the variance and featuring mechanisms in stress-induced barrier disturbance in functionally and structurally different epithelia, especially during chronological ageing. Na^+/K^+ ATPase (NKA) is universal and critical for the management of intracellular ion homeostasis and excitability[9]. It guarantees the steady-state of K^+ and Na^+ and by regulating intracellular K^+ it offers an ionic gradient for Ca^{2+} transporters. The NKA has multiple isoforms, and the dispersal of these isoforms is tissue- and development-specific, proposing that they may play specific roles, either during development or coupled to particular physiological processes[10]. The Ca^{2+} ions function as a universal messenger modulating cellular processes ranging from fertilization to apoptosis. The signalling function of Ca^{2+} requires an elaborate "toolkit" of proteins to allow Ca^{2+} influx, efflux and buffering in and between many cellular compartments and among different cells[11]. Plasma membrane Ca^{2+} ATPases (PMCAs) are highly regulated Ca^{2+} extrusion pumps that provide fine-tuning of intracellular Ca^{2+} levels.

In general, the functions of the gut are being controlled through descending connections via the hypothalamus and the amygdala; moreover, the cerebral cortex can control gut operations through direct descending connections from the infra-limbic and pre-limbic cortex to the dorsal vagus nerve complex. Hippocampus, a region of the brain conventionally linked with memory and learning and more in recent times with feeding behavior, is triggered by direct vagus nerve activation and by gastrointestinal vagus nerve-mediated signals such as the expansion of the stomach and intestinal nutrient infusion[12]. The cerebellum can influence and modify the gut functions by indirect inputs to the brainstem. It appears, consequently, that the cerebellum has more tasks than co-ordinating motor activity, showing an essential function in the coordination of somato-visceral reflexes including the integration of numerous gut functions[13]. Therefore, in this study, we explored the interaction of gut with the brain during restraint stress and ageing.

2. Materials and Methods

2.1 Animal Holding Conditions

Male Swiss albino mice (*Mus musculus*) of BALB/c strain belonging to different age groups (one month, two month, four month and six month) born and reared in the institution's animal house (University of Kerala), were used for the study. Mice were kept in groups of four each in polypropylene cages (Size: 29 x 22 x 14 cm) with stainless steel-wire mesh top. Mice were maintained in 12 hour light and 12 hour dark cycle at a room temperature of 24 ± 4ºC and relative humidity of 70 ± 10% with lowest noise levels and least handling stress. Animals were fed *ad libitum* with standard pellet feed (Sri Sai Durga Feeds and Products, Bangalore) and purified tap water. Cage bedding was replaced once every two days.

2.2 Experimental Design

Male mice of four age groups (i.e., one month, two month, four month and six month old) were used in the present study. Animals were grouped two weeks before the experiment. Special care was taken to reduce handling stress. The test groups were given restraint stress for thirty minutes for two days with a gap of twenty four hours between restraint procedures. Appropriate

control mice were also held. Proper care was taken to minimise handling stress. In this work, an age-linked response to restraint stress was analyzed in male mice. The Institutional Animal Ethical Committee (IAEC) of the University of Kerala (IAEC-KU-25/2016-17-CEIB-SP (2)) approved the experimental protocol.

2.3 Sampling and Analysis

After restraint stress, the test and control mice were euthanized by administration of Nembutal. The cortex, hippocampus, cerebellum of brain, and duodenum, jejunum, fundus and pyloric regions of the gut were dissected and kept at -80°C for further studies.

2.3.1 NKA Specific Activity

The ouabain-sensitive NKA-specific activities in the homogenates of gut tissues were quantified using the method of Peter et al. (2000) modified for microtiter plate-based assay[14]. Saponin (0.2 mg/g of protein) was added regularly to improve substrate accessibility. Samples in duplicates containing 1.0 µg protein were added to a 96-well microtiter plate containing NaCl (100 mM), imidazole (30 mM) (pH 7.4), EDTA (0.1 mM) and $MgCl_2$ (5 mM). Potassium chloride (0.13 mM) was used as promoter, and ouabain (0.14 mM) was used as inhibitor. After mixing the samples using a vortex, the assay mixture was incubated at 37°C for fifteen minutes. The reaction was triggered by adding ATP (0.3 mM) and was terminated by the addition of TCA (8.6%). The inorganic phosphate that was liberated was quantified using a phosphate standard at 700 nm in Biotek Microplate Reader (Synergy HT). The change in absorbance between promoter and inhibitor assays was calculated, and regression analysis was conducted to calculate the activity of NKA and is expressed in µM Pi h^{-1} mg $protein^{-1}$.

2.3.2 Quantification of PMCA

Briefly, about 100 mg of gut tissues were homogenized in SEI buffer (pH 7.4) and centrifuged at 1800 x g for 10 minutes. The supernatant was again centrifuged at 10000 x g for 10 minutes, and the supernatant was collected and labelled as Post-Mitochondrial Fraction (PMS). This aliquot was used to measure the specific activity of PMCA. The PMCA activity was determined as described above but vanadate was used as inhibitor[15]. Samples were added to a 96-well microplate in duplicates containing either $CaCl_2$ or vanadate. The inorganic phosphate content released was determined as above and expressed in µM Pi h^{-1} mg $protein^{-1}$.

2.4 Statistical Analysis

Before statistical analysis, data were checked for normal distribution and variance homogeneity. The statistical significance between control and test groups was tested using Two-way ANOVA followed by post-hoc Turkey test with the help of SigmaPlot 11 (Systat Software Inc.). The level of significance was accepted in the case of $p < 0.05$.

3. Results

3.1 NKA Activity

In the cortex of mice, during ageing, a significant decrease ($p<0.001$) in NKA activity was noted in two and six month old mice whereas in four months old mice a significant increase ($p<0.001$) in NKA was observed when compared to one month old mice. However, during stress, a significant increase ($p<0.001$) in NKA was observed in mice of all age groups except one month old (Figure 1A). Compared to juveniles, NKA activity in the hippocampal region of two- , four- and six month old mice showed a significant upsurge ($p<0.001$) during ageing. Restraint stress resulted in a slight increase ($p<0.01$) in NKA activity in the hippocampal region of one month old mice whereas a significant decrease was found in two-, ($p<0.001$), four- ($p<0.05$) and six month old mice ($p<0.05$) (Figure 1B). In the cerebellum of mice, compared to one month old, an increase in NKA activity was found in two- ($p<0.001$) and four month old mice ($p<0.001$) during ageing. However, in six month old mice ($p<0.001$), when compared to one month old, a significant decline was found. Restraint stress resulted in a substantial decrease in NKA activity in one ($p<0.001$), two ($p<0.01$), and four month old mice ($p<0.001$) but a slight increase was observed in six month old mice ($p<0.01$) (Figure 1C).

In the fundus, no significant change was found in the NKA activity during ageing but restraint stress caused an enhanced activity in one month old mice ($p<0.001$) whereas a decline was found in two ($p<0.001$), four ($p<0.01$) and six month old stressed mice ($p<0.001$) (Figure 2A). In the pyloric stomach, during ageing, a significant decrease in NKA activity was found in two ($p<0.001$), four ($p<0.001$) and six month old mice

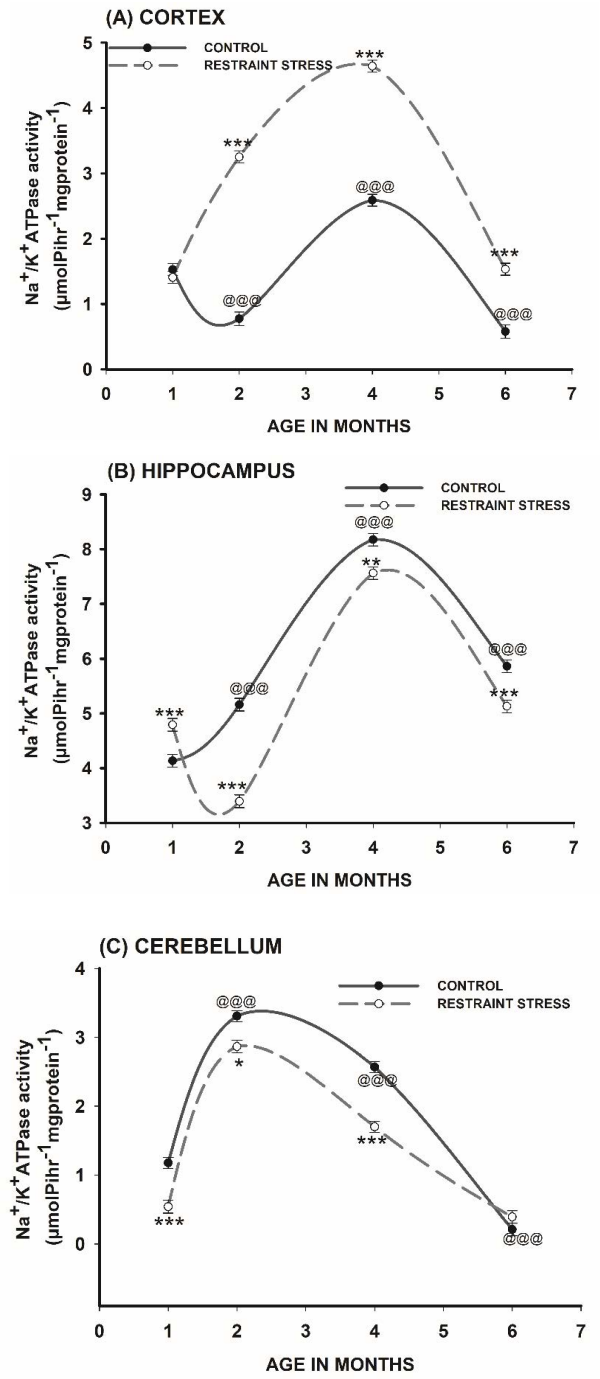


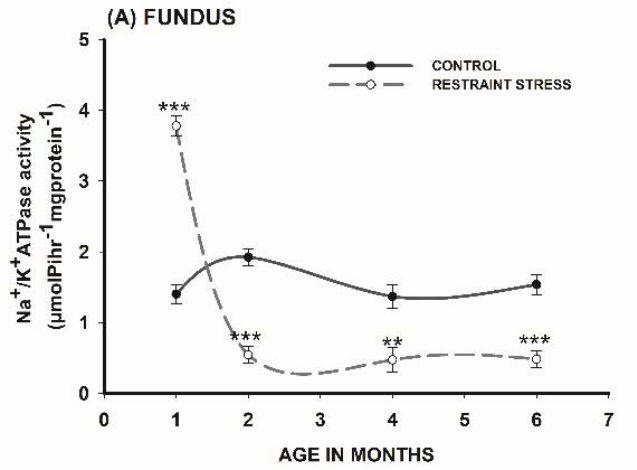
Figure 1. Activity pattern of Na+/K+-ATPase in the cerebral cortex (A), hippocampus (B) and cerebellum (C) during ageing and restraint stress in male mice. Each point is mean ± SE for four mice. The significance is represented as "*" for restraint stress (compared to control) and "@" for ageing (compared to 1-month old mice). Levels are "*" "@" ($p < 0.05$), "**" "@@" ($p < 0.01$) and "***" "@@@" ($p < 0.001$).

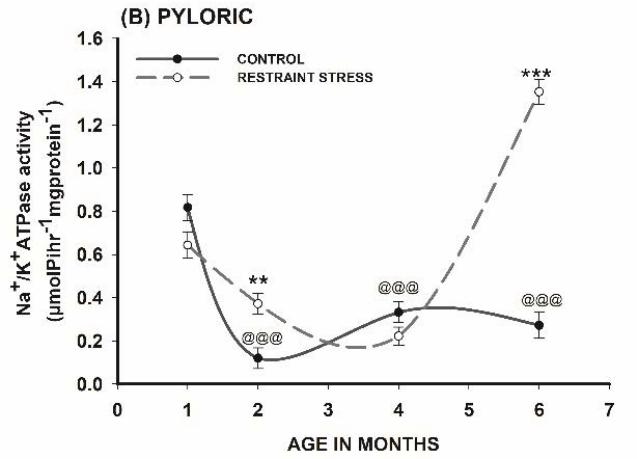
($p<0.001$) compared to one month old. Stress raised the NKA activity of six month old mice ($p<0.01$) whereas no significant change was observed in response to stress in one, two and four month old mice (Figure 2B). In the duodenum, no age-dependent change in NKA activity was found but during stress a surge in NKA activity was found in two ($p<0.05$) and four month old mice ($p<0.01$) whereas no significant difference was noticed in the response to stress in one and six month old mice (Figure 2C).

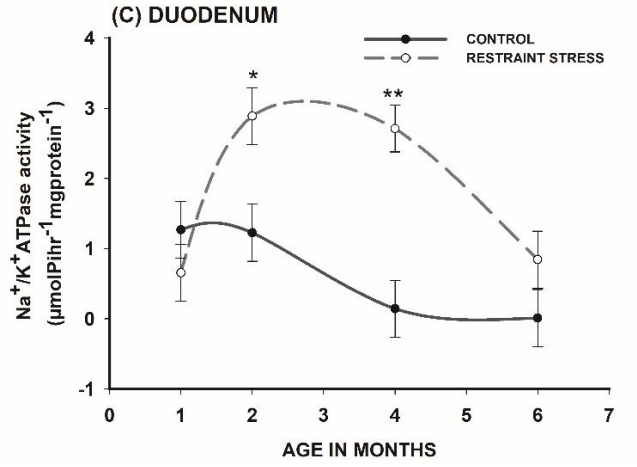
In the jejunum, compared to one month old mice, an increase in NKA activity was found in two ($p<0.001$) and four month old mice ($p<0.001$) whereas no significant change was observed in six month old mice compared to one month old. Restraint stress resulted in an increase in activity in one month old mice ($p<0.001$) whereas in two month old mice ($p<0.05$) a decrease in NKA activity was found during stress. In four and six month old mice no significant difference was documented in the activity after stress (Figure 2D).

3.2 PMCA Activity

In the cortex of mice, a significant increase ($p<0.001$) in PMCA activity was found in all age groups under study except one-month-old mice. After restraint stress, an increase in PMCA activity was found in one ($p<0.001$), and four month old mice ($p<0.001$) whereas stress resulted in a substantial reduction in PMCA activity of two ($p<0.001$) and six month old mice ($p<0.001$) (Figure 3A). The PMCA activity of the hippocampus showed a significant increase in two ($p<0.001$), four ($p<0.01$) and six month old ($p<0.001$) compared to one month old mice. Restraint stress resulted in a substantial increase in PMCA activity of hippocampus in mice of all age groups, i.e., one month ($p<0.001$), two month ($p<0.001$), four-month ($p<0.01$) and six month ($p<0.001$) (Figure 3B). In the cerebellum of mice, compared to one month old mice, an increase in PMCA activity was found in four month old mice ($p<0.001$) whereas a significant decline was observed in two ($p<0.001$) and six month old mice ($p<0.001$) during ageing. Restraint stress resulted in a significant decrease in PMCA activity in one month ($p<0.001$) and four month old mice ($p<0.001$) whereas a significant increase was observed during the stressed condition in two ($p<0.001$) and six month old mice ($p<0.001$) (Figure 3C).







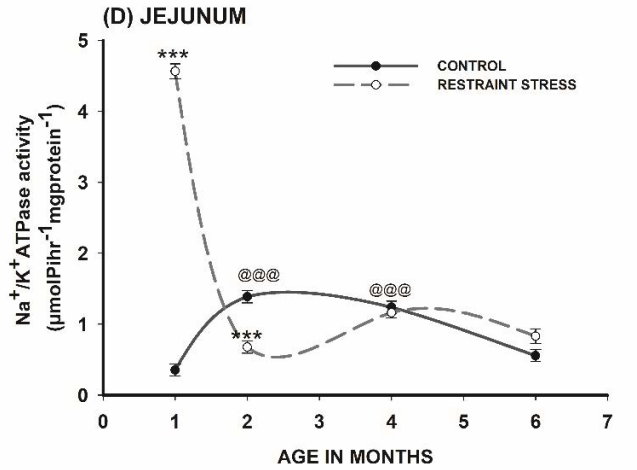


Figure 2. Activity pattern of Na+/K+-ATPase in the fundus (A), pylorus (B) duodenum (C) and jejunum (D) during ageing and restraint stress in male mice. Each point is mean ± SE for four mice. The significance is represented as "*" for restraint stress (compared to control) and "@" for ageing (compared to 1-month old mice). Levels are "*" "@" ($p < 0.05$), "**" "@@" ($p < 0.01$) and "***" "@@@" ($p < 0.001$).

In the fundus, during ageing, a significant decline in PMCA activity was found in two ($p<0.001$), four ($p<0.001$) and six month old mice ($p<0.001$) compared to one month old. Stress resulted in a decreased PMCA activity in one month ($p<0.001$) and two month old mice ($p<0.001$) but an increase was found in four month old mice ($p<0.001$). No statistically significant change was observed in the PMCA activity of six month old mice in response to stress (Figure4A). In the pyloric region, when compared to one month old mice, a decrease was found in two month old mice ($p<0.001$) whereas an increase was found in four month old mice ($p<0.001$). However, no significant variation was found in the PMCA activity of six month old mice compared to one month old. Restraint stress resulted in an increase in PMCA activity in two ($p<0.001$) and six month old stressed mice ($p<0.001$), but a decrease was found in four month old mice ($p<0.001$). No significant change was found in stress-exposed one month old mice (Figure 4B). In the duodenum, ageing resulted in a decline in PMCA

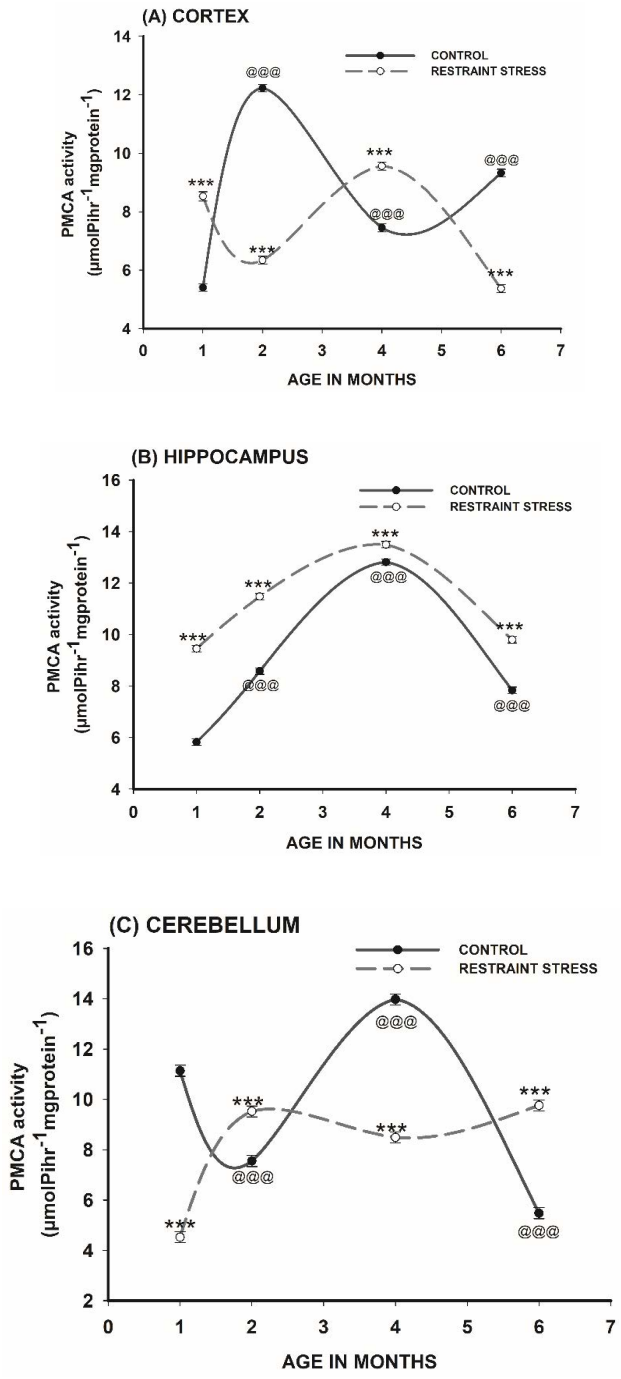


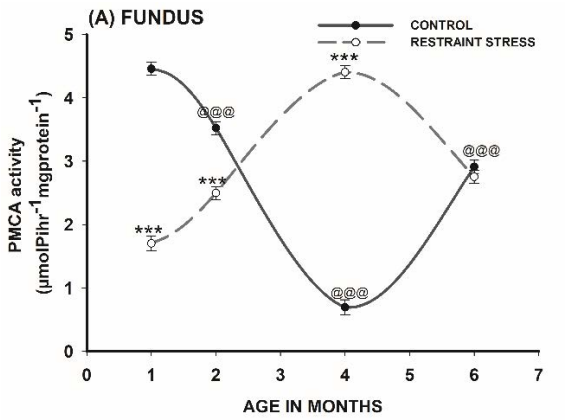
Figure 3. Activity pattern of PMCA in the cerebral cortex (A), hippocampus (B) and cerebellum (C) during ageing and restraint stress in male mice. Each point is mean ± SE for four mice. The significance is represented as "*" for restraint stress (compared to control) and "@" for ageing (compared to 1-month old mice). Levels are "*" "@" ($p < 0.05$), "**" "@@" ($p < 0.01$) and "***" "@@@" ($p < 0.001$).

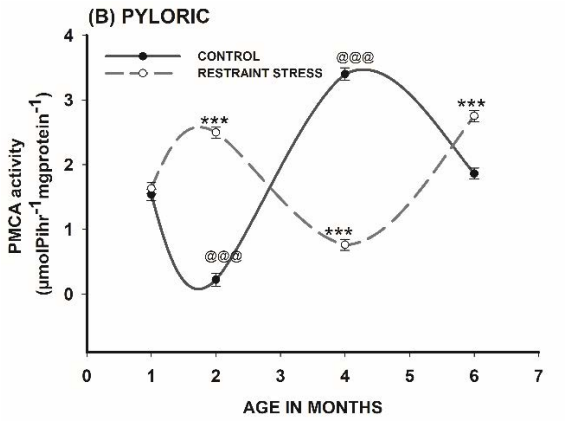
activity in two ($p<0.001$), and month old mice ($p<0.001$) whereas no significant difference was found in six month old mice. During the stressed condition, an increase in PMCA activity was observed in two ($p<0.001$), four - ($p<0.001$) and six month old mice ($p<0.001$). However, no significant difference was found in one month old mice as a result of restraint stress (Figure 4C). In the jejunum, a decline in PMCA activity was observed during ageing in two ($p<0.001$), four ($p<0.001$) and six month old mice ($p<0.01$) compared to one month old pups. In response to stress, a significant increase in PMCA activity was found in one month ($p<0.05$) and a decrease was found in two ($p<0.001$) and four month old mice ($p<0.001$) whereas no significant difference was found in six month old mice (Figure 4D).

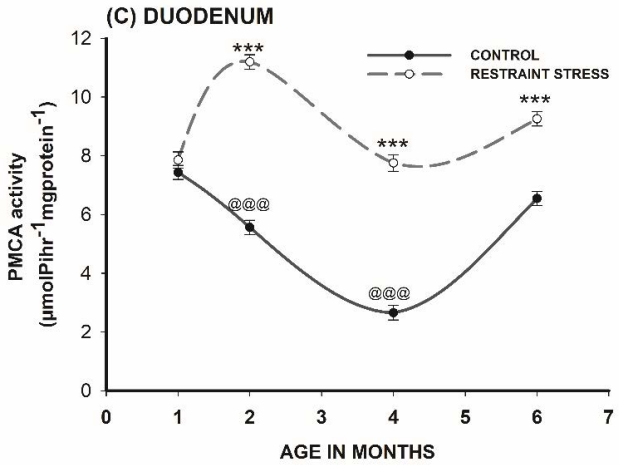
4. Discussion

Stress as an internal or external stimulus is capable of disrupting the physiological environment, and the ability of an organism to handle such aversive situations is a critical contributing factor to health and disease[16]. Stress has an effect on the lifespan and quality of ageing[7]. The notion that the gut and the brain are closely associated, and that this association plays a crucial part not only in gut function but also in individual feeling states and intuitive decision making. Neurobiological investigations in the gut-brain interactions have revealed a complex, two-way communication system that not only guarantees proper preservation of gut homeostasis and digestion but also is expected to have various effects on motivation and advanced cognitive functions, like intuitive decision making[17]. The data gathered in experimental conditions using model organisms reveal that disturbances in the brain, such as those caused by physical or psychological stress, can affect gut function. Moreover, variations of the gut microenvironment can persuade behavioral and neurochemical vagaries. Hence, it is possible to explore 'brain-to-gut' as well as a 'gut-to-brain' regulation[18].

Na^+/K^+-ATPase (NKA) is an enzyme accountable for producing and upholding the ionic gradients essential for neuronal excitability[19]. In the present study, an age-dependent differential pattern was observed in the NKA activity of the cortical region, and the activity reaches its zenith in young adults. After restraint stress, an upsurge was detected in NKA activity of cerebral cortex of mice of all age groups except juveniles. In many studies,







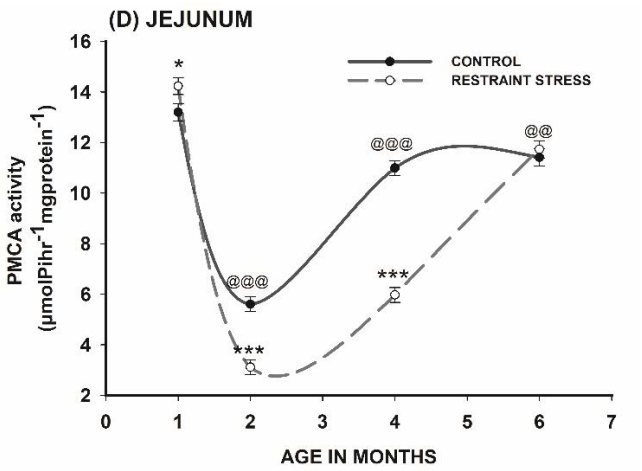


Figure 4. Activity pattern of PMCA in the fundus (A), pyloric (B) duodenum (C) and jejunum (D) during ageing and restraint stress in male mice. Each point is mean ± SE for four mice. The significance is represented as "*" for restraint stress (compared to control) and "@" for ageing (compared to 1-month old mice). Levels are "*" "@" ($p < 0.05$), "**" "@@" ($p < 0.01$) and "***" "@@@" ($p < 0.001$).

the relationship between NKA activity and the release of neurotransmitters has been validated, proposing that NKA plays a functional role in the modulation of neurotransmission[19]. Studies have shown that different stressors increase the release of dopamine, noradrenaline, serotonin and acetylcholine in the areas of the brain[20]. The never-ending firing of nerve impulses during stressed condition requires very high activity of NKA for the conservation of the ion gradients in the nerve cell in the cortex[21]. In this work, an increase in NKA activity was found during ageing and the highest activity was observed in young adults. However, NKA activity was lowered in the hippocampal region of adolescents, young adults and mature adult mice that were exposed to restraint stress. These results help to associate alterations in behavior with biological modifications of neurons primarily at the neuro-chemical level, which is a significant finding in an animal model of disease. Hippocampus complex

is activated during stress, primarily by noradrenergic neurons ,and is essential for retrieval and emotional analysis of information pertinent to the stressor[22]. In the cerebellum, NKA activity of adolescents and young adults showed an upsurge when compared to juveniles but the activity showed a decline in mature adults during ageing. However, in mice exposed to restraint stress, a substantial decline in NKA activity was observed in juveniles, adolescents and young adults whereas no significant stress response was observed in mature adults. The rise and decline in NKA activity may be supposedly linked to neuroprotective or cell death-promoting activities. In chronically depolarised neurons, increased NKA activity could be seen to decrease intracellular Na^+ levels to assist the restoration of cellular homeostasis, but the increased demand for ATP could be harmful. On the other hand, reduced NKA activity in injured cells may allow augmented intracellular Na^+, but the lessened demand for ATP from less active NKA may be neuroprotective[23].

Regulation of NKA activity is a complex phenomenon, and it seems to be organised by numerous aspects comprising of neurotransmitters and hormones such as catecholamines and serotonin. It should be noted that central catecholaminergic and serotonergic activities might be modulated by contact with stressors[24]. There is substantial evidence for the governing role of norepinephrine on the activity of NKA in the brain. Moreover, brain NKA is differentially regulated by serotonin (5-HT) in many areas of normal rat brain *in vivo* and *in vitro*[25]. A functional correlation was found between NKA and these neurotransmitter amines. Monoamines can either stimulate or inhibit NKA by receptor-mediated mechanisms and, as a result, regulate the excitability of neurons[26].

In this study, NKA activity in the fundus of mice shows no change in activity with increasing age; however, in response to stress a decline in NKA activity was found in adolescents, young adults and mature adults but a surge was found in juveniles. In the pyloric region of mice during ageing, a decline in NKA activity was found in all age groups under study. Restraint stress resulted in no significant change in NKA activity in juveniles and young adults but a significant upsurge was found in adolescence and mature adult. The NKA plays a vital role in the movement of ions, nutrients, and water across the intestine. By creating a sodium and potassium gradient across the enterocyte membrane, it provides the driving force needed for the activity of many symporters and antiporters and the sodium-coupled transport of sugars, amino acids and other molecules. Thus, any change in the activity of the intestinal NKA will result in either an enhancement or an impairment of these transport processes[27]. In this study, no significant change in NKA activity was observed in the duodenum during ageing but restraint stress resulted in an age-specific differential response in NKA activity. In the jejunum, NKA activity was higher in adolescent and young adults than juveniles and mature adults. Restraint stress resulted in an increase in activity in juveniles, whereas in adolescents, a decrease in NKA activity was noted during stress. In young and mature adult males, no significant difference was documented in the activity during stress. Jejunum constitutes an essential site of nutrient absorption, and the NKA plays a crucial role in the regulation of this absorption[27]. NKA is prominent in numerous primary and specialized cellular activities, and this enzyme must be able to acclimatise to altering cellular and physiological impetuses. Multiple mechanisms can probably control the operation of NKA exposed to either physiological or pathophysiological conditions[28].

The ion gradients generated by the NKA also regulate the concentration of ions such as Ca^{2+}. This is further supported by studies which showed that inhibition of the NKA by ouabain caused an upsurge in contractile force, which is thought to be due to the surge in Ca^{2+} levels through the Na^+/Ca^{2+} exchanger. When cellular Na^+ increases, the Na^+/Ca^{2+} exchanger acts in the opposite direction and transports Ca^{2+} into the cell. The increased calcium ions then work to enhance muscle contraction[29].

The Ca^{2+} functions as a signal for the modulation of several neuronal purposes like membrane excitability, neurotransmitter discharge and Ca^{2+} influx. Moreover, Ca^{2+} is essential for activating depolarization-evoked neurotransmitter discharge. Under physiological conditions, calcium homeostasis in neurons is upheld by a finely conserved interplay between calcium influx and releasing channels called the Ca^{2+} channels, and calcium efflux mechanisms like Ca^{2+}-pumps and Ca^{2+}-exchangers. The principal functional modules of the calcium efflux machinery are the Plasma Membrane Ca^{2+}-ATPases or simply PMCAs, which embody high-affinity calcium pumps accountable for the removal of calcium out of the cytosol in an ATP-dependent manner. The function of the PMCAs in the central nervous system is the precise restoration of the basal-line levels of intracellular Ca^{2+} after its transient raise during calcium signalling[30].

In the cortex, compared to juveniles, an increase in PMCA activity was found during ageing but after restraint stress, an upsurge in PMCA activity was prominent in juveniles, and young adults and a reduction in PMCA activity were observed in adolescents and mature adults. The upsurge in PMCA activity suggests that juveniles and young adults have a more effective Ca^{2+} efflux mechanism than adolescents and mature adults. Reports from previous studies indicate that acute restraint stress considerably increases the resting intracellular Ca^{2+} concentration in cortical synaptosomes of the cerebrum in mice. The cortical areas are known to have a crucial function in the storing of long-term memory[31]. Literature is available showing that Ca^{2+} can act as stress-mediator and its function in scheming stress-induced behavioral changes and roles in memory weakening[32]. In this study, a differential stress response was found in the cortical region concerning age. In the hippocampus of mice, an increase in PMCA activity was noted in adolescents, young adults and mature adults when compared to juveniles. During restraint stress, a surge in PMCA activity was found in all the experimental groups in response to restraint stress. In the cerebellum of mice, PMCA showed an age-linked differential activity. During stress response, a fall in activity was detected in juveniles, and young adults and an elevation after restraint stress were found in adolescents and mature adults. The cerebellum is the region of the brain mainly involved in motor control and sensory perception. The PMCAs are accountable for accurate regulation of local Ca^{2+} at excitatory parallel fiber-Purkinje nerve cell synapse terminals, thereby controlling neurotransmitter discharge[33]. These pumps are hence considered as significant caretakers of general Ca^{2+} homeostasis and help in spatially and temporally distinct Ca^{2+} signalling events[34]. Age-related changes in calcium homeostasis have also been well-documented[30].

The gut is the lone gate for the entrance of Ca^{2+} to the body in mammals. Gastrointestinal Ca^{2+} absorption is a necessary process that occurs through an active trans-cellular pathway which is structured by hormones, nutrients and many other factors. This mechanism is energy-dependent, and associates Ca^{2+} movement from the mucosal to serosal side of the intestinal barrier stirring against a concentration gradient[35]. The homeostasis of intracellular Ca^{2+} is organised by a range of ion channels and proteins[36]. Plasma membrane Ca^{2+}-ATPase is an ATP-dependent transporter that pumps Ca^{2+} out of the cytosol. Various studies indicate that during ageing several aspects of Ca^{2+} homeostasis might be affected, as Ca^{2+} influx, the release of Ca^{2+} from intracellular spaces and Ca^{2+} uptake processes by the sarcoplasmic reticulum and mitochondria[37]. The present study aimed at characterizing the changes in the intracellular Ca^{2+} stores during stress response in the gastrointestinal smooth muscle from juveniles to mature adults. The results showed that ageing might alter Ca^{2+} stores, and a differential pattern with respect to age was observed in response to stress. These changes might affect smooth muscle contractility after restraint stress and may be linked to stress-induced gastrointestinal disorders like gastric paresis. Different regions of the gut presented different patterns in Ca^{2+}-ATPase activity in response to restraint stress, and this could be correlated with their differential potential of Ca^{2+} absorption. The chief contributors to the amount of Ca^{2+} absorption are the residence time and the absorption rate in each segment of the intestine. The order of Ca^{2+} absorption rate is the greatest in duodenum followed by jejunum. Intestinal Ca^{2+} absorption also depends on the physiological needs of Ca^{2+}. When the requirements rise, and the intakes are low, there is an improvement in the efficiency of Ca^{2+} absorption[38].

This study presents physiological evidence for a differential response of ion transporter activity during ageing and restraint stress in the brain and gastrointestinal tract of mice. The same ion-transporter showed different stress responses in various stages of life in a tissue-dependent manner. Moreover, this finding provides support to the concept that gastrointestinal and neurological functions are closely associated during the stress response. A stress response is usually transient and physiologically significant for existence, to adjust with a changing environment or to handle hypothetically life-threatening circumstances in many organisms[39]. Recognition of the physiological mechanisms to manage stress is the key to upholding healthy aftermaths following stress. This study thus provides insight on the physiological mechanism of the brain-gut axis to both ageing and restraint stress

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6. References

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