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Immobility Responses Affected by Potassium in Old Rats

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Abstract

Four immobility responses (IR): elicited by clamping, bandaging, grasping and inversion, and their modification by potassium and spironolactone was studied in old Wistar rats (body weight, 500 g). When undrugged, only clamping and grasping, but not bandaging and inversion induced an IR in rats. Potassium and spironolactone significantly enhanced the duration of IR induced by clamping but not by grasping. They also induced an immobility response by bandaging, but not by inversion. The data suggest that IR induced by clamping and bandaging are somehow related to changes in the potassium serum levels. Consequently, such a relationship may be a suitable model to study some forms of paralysis in human beings which are related to changes in the potassium serum levels.

Keywords

Immobility Responses (IR), Potassium, Spironolactone, Hyperkalemia, Familial Periodic Paralysis

1. Introduction

The immobility response (IR) is probably better known as "animal hypnosis", a state of profound immobility and relative unresponsiveness, which can be triggered in many invertebrate and vertebrate species. Most of the IR induction methods involve placing the animal into an awkward posture that is temporarily sustained by manual restraint. The IR typically ensues as a response or reaction

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to a constellation of tactile, proprioceptive, vestibular and sometimes visual stimuli [1]. Treatment with drugs, particularly dopaminergic blockers or opiates is powerful in potentiating IR [2] [3]. Differences in this response among species may result from differential susceptibility to the induction of IR by different stimuli. While all induction methods have certain features in common, there are subtle differences between them that may offer important clues as to the mechanisms involved in the induction of IR. Tonic immobility (TI) is induced by placing an animal on its back. Such a state is easily induced in guinea pigs [4], rabbits, and frogs, but not in an adult rat [1]. However, tonic immobility is readily exhibited in newborn rats, and its duration is potentiated with haloperidol, a dopamine antagonist at 10 days of age but not older [5].

Another kind of immobility is the dorsal immobility response (DIR), in which the animal is grasped by the skin of the dorsal surface of the neck and lifted into the air [5] [6] [7] [8]. DIR is easily induced in rabbits, chickens, guinea pigs and rats. Haloperidol in 20-day old rats potentiates DIR but not TI [5]. Haloperidol enhances the duration of DIR in adult rats [3] [5], but in infant rats has the opposite effect [9].

The IR can also be elicited by clamping (IC) the nape of the neck and placing the animal on its back [10] [11] [12]. The animal remains immobile in a ventroflexed posture, with its limbs tucked into its body. The effective stimulus for these responses is tactile pressure on the skin of the nape which can be achieved not only by an experimenter's finger or by clamping but also by an adult rat's teeth carrying neonate and young rats [11] [13]. Neonate rats are very susceptible to IR, since IR can also be elicited by placing a bandage around the head and neck, known as (IB) "bandaging" [11]. Adult rats continue to exhibit the DIR, as well as the IR elicited by clamping, but not by bandaging [11].

On the other hand, there is a connection between some forms of paralysis (an immobility form) in human beings and changes in the potassium serum levels. For instance, the "familial periodic paralysis" (FPP) is a disability characterized by an alteration in the sodium channels, as well as alterations in potassium levels. This disease is characterized by sporadic attacks of weakness and flaccid muscle. Moreover, it can show two forms: the hypokalemic [14] and the hyperkalemic [15]. These kinds of diseases can be induced by mutations, the diet, the environment and the routines of the individual.

The FPP induced by hypokalemia (Hypo PP) is characterized by levels of serum potassium less than 3.5 mEq/L; it is characterized by episodes of weakness, fatigue, and in some cases myotonia, especially in eyelids. The episodes of paralysis in Hypo PP are usually evident in puberty; between the ages of 15 and 35 years old [16]. Published case series suggest the highest frequency of attacks in teenagers followed by a decreasing attack frequency in line with increasing age in both HyperPP and HypoPP [17] [18]. Hypo PP is associated with autosomal dominant genes that induce mutations in the sodium and calcium channel genes [18] [19]. Moreover, this disease can be triggered by environmental factors such as rich-carbohydrate diets, barium poisoning [20] [21] [22] [23], certain disord-

ers such as in the renal dysfunctions related to changes in potassium levels [24] [25], and the use of diuretics [26] [27] [28]. Cases of thyrotoxic periodic paralysis (TPP) have also been reported, which is induced by an increase in the levels of thyroid hormones, which trigger episodes of hypokalemia [29].

The FPP induced by hyperkalemia (Hyper PP) is characterized by levels over 4.5 mEq/L. The episodes in this disease are usually more frequent in infancy [16]. As Hypo PP, Hyper PP shows signs of muscle weakness. However, it also presents muscle stiffness, and myotonia. The duration of the paralysis in Hyper PP is less than 2 hours. In addition, the attacks in Hyper PP are more frequent and shorter than Hypo PP [30]. Unlike Hypo PP, Hyper PP is just associated with mutation in sodium channels [19] [20], however it is also triggered by environmental or external factors. These factors can trigger hyperkalemia, such as the intake of rich-potassium food (bananas, melons, potatoes, avocados, and citric juices; shifting in cellular potassium levels; a decrease in renal excretion [24] [25]; and insulin deficiency [31]. Moreover, the substitutes of sodium, laxatives, beta adrenergic blockers, inhibitors of the renin-angiotensin-aldosterone system, such as spironolactone, and diuretics can trigger hyperkalemia, as well as habits such as fasting, stress and cold temperatures. There are even reports that patients ate substances which are not food like the head of used matches [32] or red clay [33]. There have also been cases of pseudohyperkalemia, which is associated with leukemia, thrombocytosis, and renal disease [34] [35] [36], and reverse hyperkalemia, which is associated with leukemia [37] [38] [39] [40] [41].

The diagnosis for Hypo PP or Hyper PP are elaborated by the signs and symptoms in the patient, such as the serum level of potassium at the moment of the attacks, the presence of triggers as were mentioned before, cases in other members of the family and the evidence of genetic mutations related to changes in potassium levels. These disorders can trigger renal and cardiac diseases; they can even reduce the quality of life of patients who get these kinds of alterations [17] [18] [42] [43]. In both forms, paralysis is the most conspicuous symptom, the pathophysiology of which is not well understood [17] [44]. Therefore, the objective of this study was to investigate whether serum potassium levels, either by direct potassium administration or by indirectly modifying their levels by the administration of an aldosterone receptor blocker, have an effect on the duration of the immobility response elicited by four methods in rats.

2. Methods

All experimental procedures in this study have followed the guidelines of the Mexican government (NOM-062-ZOO-1999), which details the technical specifications for production, care, and use of laboratory animals. The internal Bioethics Committee approved this protocol.

Fifty male adult Wistar rats (505 ± 10 g) were used, provided by the bioterium of the National School of Biological Sciences. All animals were individually housed, kept and tested at a temperature of 21˚C ± 1˚C, and 12:12 h light/dark cycle, with lights on at 07.00 h. Experimental testing began between 10.00 and

11.00 h. The behavior was recorded on videotape for their posterior analysis.

2.1. Procedure

The following substances were administered intraperitoneally (i.p.): potassium chloride (KCl: Sigma-Aldrich) 300 and 500 mg/kg or sodium chloride (NaCl: Sigma-Aldrich) 0.9% and 500 mg/kg. And intragastrically: Spironolactone (Aldactone, Searle) 75 mg/kg. Groups of ten randomly selected rats were used for each treatment. Animals were tested just prior to injection and then each hour for the next 3 h. Preliminary dose-response experiments in male rats of different weights (200 ± 10, 300 ± 10, and 505 ± 10) were conducted to determine the possible relations between body weight, treatment, and dose. Since rats that weighed 200 or 300 g did not respond to any treatment, such data are not shown. All drugs were administered in volumes of 3 mL/kg. Subsequently, other groups of rats were administered with the doses that showed an effect such as NaCl 300 mg/kg and 500 mg/kg; KCl 300 mg/kg and 500 mg/kg, and two doses of spironolactone (50 mg/kg, and 150 mg/kg) to observe the effects of these substances on the immobility responses. Because of KCl 500, mg/Kg showed the most persistent effects, we decided to test this dose on groups of old and young rats to test if there were differences between them.

The behavioral tests were recorded on videotape using a VHS video camera (NV-N3000PN, Panasonic) for later analysis.

2.2. Behavioral Testing

The immobility response (IR) was induced in all animals by the following 4 methods in the order described below. The methodology has been well established and described in previous works [2] [3] [11]. In each method of induction, all animals received four trials (0, 1, 2, and 3) with an intertrial interval of 1 h.

2.2.1. Inversion (Also Called Tonic Immobility TI)

To induce tonic immobility, the animal was rapidly inverted and restrained on its back for 3 s. before removing the hand holding the imposed flank. Measurement of the duration of tonic immobility began with the release of the subject and ended when the animal returned to a prone position, standing on all four paws or until 180 s had elapsed.

2.2.2. Clamping the Neck (IC)

A clamp was applied between the base of the skull and the back of the ears, the pressure being sufficient to lift the whole animal by the clamp. The animal was then placed on a flat surface, on its side. The duration of immobility was measured from the moment it was placed on its side until the animal recovered the prone position, or to a maximum of 180 s.

2.2.3. Bandaging the Neck (BI)

A bandage was snugly wrapped around the head and neck (but not tight enough as to impair breathing). The animal was then placed on its side onto a flat sur-

face. The duration of immobility was measured as above.

2.2.4. Grasping the Neck (Also Called Dorsal Immobility DIR)

The animal was gently grasped by the nape of the neck between the base of the skull and the back of the ears, and was lifted off its feet, so that no part of the animal's body touched any other surface. The duration of immobility was measured from the onset of the response (which was instantaneous), until the animal made escape-like movements directed at the experimenter's hand, or until 180 s. had elapsed.

2.3. Analysis

Data were analyzed using an analysis of variance for Repeated Measures on Ranks and the posteriori test (Tukey) in which comparisons of each treatment against the saline control values were made (0.9%). $^{*}p < 0.05$; $^{**}p < 0.01$

3. Results

The results for immobility by inversion are not shown because this kind of immobility was not presented in any time with any treatment.

Figure 1 shows the results for the duration of the immobility response induced by clamping. There was a significant increase in the duration of the immobility elicited by clamping, at 1, 2 and 3 hours after the administration of KCl 500 mg/kg and spironolactone 75 mg/kg ($F_{(3)} = 9.693$, $P < 0.001$). However, NaCl 500 mg/kg ($P < 0.857$) and KCl 300 mg/kg ($P < 0.252$) did not increase the duration of immobility induced by clamping versus the basal time (0 h). There were also differences between the treatments and the interaction (treatment x time) ($F_{(4)} = 4.573$, $P < 0.003$ and $F_{(12)} = 3.286$, $P < 0.001$ respectively). The immobility response also was modified by the different substances administrated. Within

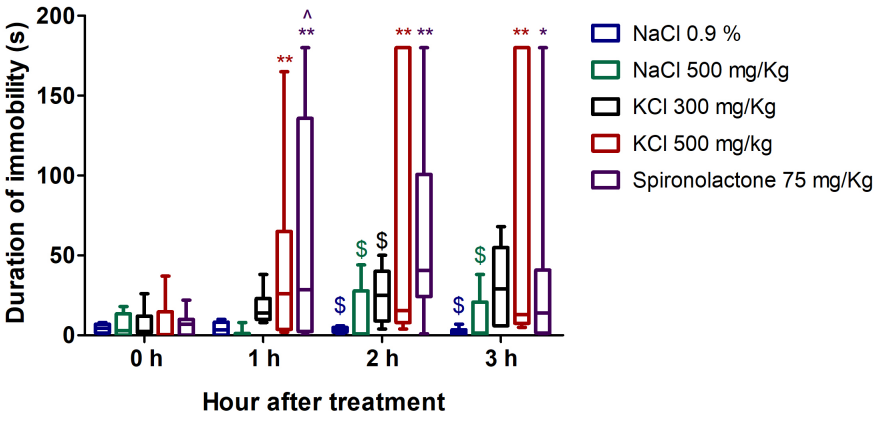

Figure 1. Effects of NaCl 0.9% (control), NaCl 500 mg/kg, KCl 300 mg/kg, KCl 500 mg/kg and spironolactone 75 mg/kg on the duration of immobility response induced by clamping. The boxes and whiskers represent the median plus the maximum and the minimum value. $^{**}P < 0.01$ vs time at 0 h (basal); $^{\wedge}P < 0.01$ vs NaCl 0.9% and 500 mg/kg, KCl 300 mg/kg.; $^{\circ}P < 0.01$ vs spironolactone; $^{\$}P < 0.01$ vs KCl 500 mg/kg. Two way RM ANOVA, post hoc Tukey.

the first hour (1 h) after treatment, the administration of spironolactone 75 mg/kg significantly increased the duration of immobility by clamping versus treatments with NaCl 0.9% ($P < 0.004$); NaCl 500 mg/kg ($P < 0.002$); KCl 300 mg/kg ($P < 0.04$), but there was no difference versus KCl 500 mg/kg ($P < 0.526$). Within the second hour (2 h) after treatment, the administration of spironolactone 75 mg/kg and KCl 500 mg/kg increased the duration of immobility by clamping. Spironolactone 75 mg/kg was different versus NaCl 0.9% ($P < 0.006$) and 500 mg/kg ($P < 0.024$); and KCl 500 mg/kg was different versus NaCl 0.9%, NaCl 500 mg/kg, and KCl 300 mg/kg. And within the third hour (3 h) after treatment, KCl 500 mg/kg augmented the duration of immobility regarding NaCl 0.9% and NaCl 500 mg/kg.

The immobility induced by bandaging (as we explained before) is not present in adult rats. However, the administration of KCl 500 mg/kg ($F_{(4)} = 2.595$, $P < 0.049$) induced the immobility by bandaging in adult rats (Figure 2), it was different between time 0 h (basal) versus the third hour (3 h) after treatment ($P < 0.009$). KCl also showed differences within the second hour (2 h) after treatment versus NaCl 0.9% ($P < 0.032$) and KCl 300 mg/kg ($P < 0.047$).

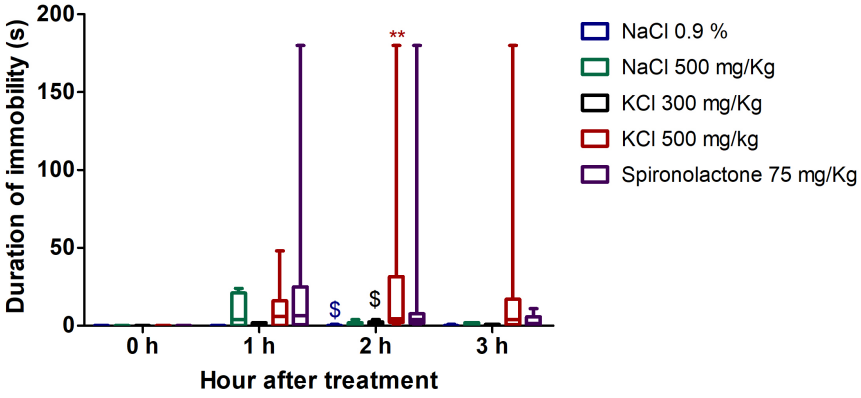

Figure 2. Effects of NaCl 0.9% (control), NaCl 500 mg/kg, KCl 300 mg/kg, KCl 500 mg/kg and spironolactone 75 mg/kg on the duration of immobility response induced by bandaging. The boxes and whiskers represent the median plus the maximum and the minimum value. $^{**}P < 0.01$ vs time at 0 h (basal); $^{\$}P < 0.01$ vs KCl 500 mg/kg. Two way RM ANOVA, post hoc Tukey.

Figure 3 shows the duration of immobility by grasping. This kind of immobility is presented in adult rats and we found that it is modified by the time ($F_{(3)} = 5.432$, $P < 0.001$); the treatment ($F_{(4)} = 6.165$, $P < 0.001$) and the interaction time x treatment ($F_{(12)} = 2.59$, $P < 0.004$). KCl 300 mg/kg augmented the duration of immobility by grasping at time 1 h after treatment ($P < 0.001$) and at 3 h after treatment the duration decreased ($P < 0.001$). Spironolactone 75 mg/kg augmented the duration of immobility 2 hours after treatment ($P < 0.001$) and then the immobility decreased at 3 h after treatment ($P < 0.043$). Within the first hour (1 h) after treatment, KCl 300 mg/kg increased the duration of immobility significantly versus NaCl 0.9% ($P < 0.001$), NaCl 500 mg/kg ($P = 0.001$) and KCl

500 mg/kg ($P < 0.001$). Within the second hour after treatment, spironolactone 75 mg/kg significantly increased the immobility versus the treatments of NaCl 0.9% ($P < 0.001$), NaCl 500 mg/kg ($P < 0.001$), and KCl 500 mg/kg ($P < 0.001$).

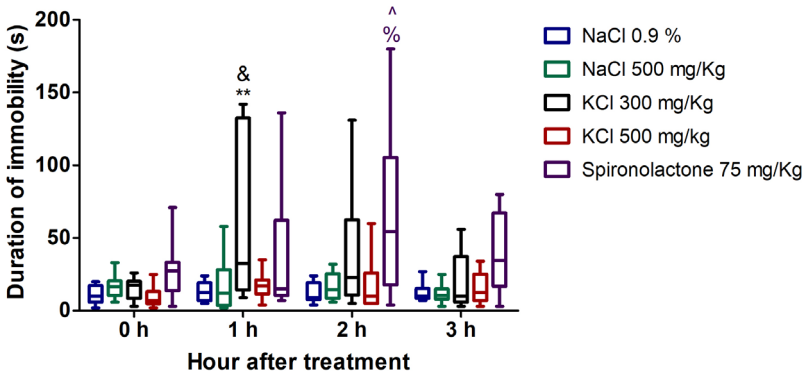

Figure 3. Effects of NaCl 0.9% (control), NaCl 500 mg/kg, KCl 300 mg/kg, KCl 500 mg/kg and spironolactone 75 mg/kg on the duration of immobility response induced by grasping the boxes and whiskers represent the median plus the maximum and the minimum value. $^{**}P < 0.01$ vs time at 0 h (basal) and 3 h after treatment; $^{\%}P < 0.01$ vs time 0 h (basal), 1 h and 3 h after treatment; $^{\&}P < 0.01$ vs NaCl 0.9%, 500 mg/kg and KCl 500 mg/kg; $^{\wedge}P < 0.01$ vs NaCl 0.9%, 500 mg/kg and KCl 500 mg/kg . Two way RM ANOVA, post hoc Tukey.

After testing the previous substances in different doses, we used KCl 500 mg/kg because this salt and this dose presented the most consistent effects. KCl 500 mg/kg augmented the immobility response induced by clamping (**Figure 4**) in old rats (time: $F_{(3)} = 3.185$, $P < 0.031$ and the interaction: time × age, $F_{(3)} = 3.608$, $P < 0.019$) from the second hour (2 h) ($P < 0.002$) and remained until the third hour (3 h) ($P < 0.014$) after treatment. KCl 500 mg/kg also had a different effect increasing the duration of immobility response by clamping in old rats compared to the young rats within the second hour ($P < 0.004$) and remained until the third hour ($P < 0.02$).

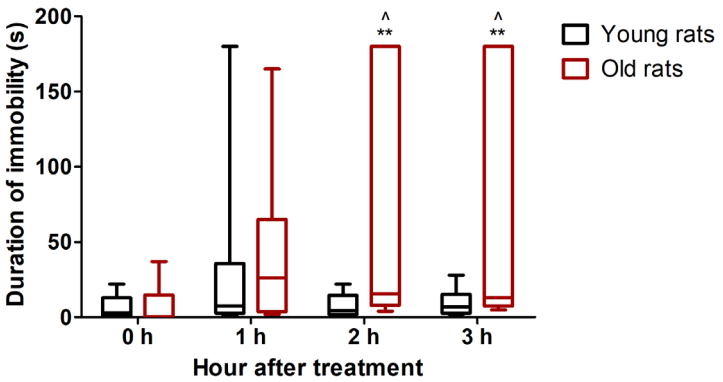

Figure 4. Effect of KCl 500 mg/kg on the duration of immobility response induced by clamping in young and old rats. The boxes and whiskers represent the median plus the maximum and the minimum value. $^{**}P < 0.01$ vs its basal (0 h); $^{\wedge}P < 0.01$ vs young rats. Two way RM ANOVA, post hoc Tukey.

However, the duration of immobility induced by bandaging (Figure 5: $F_{(3)} = 1.47$, $P < 0.23$) and by grasping (Figure 6: $F_{(3)} = 1.816$, $P < 0.155$) was not modified in old rats compared with the young rats.

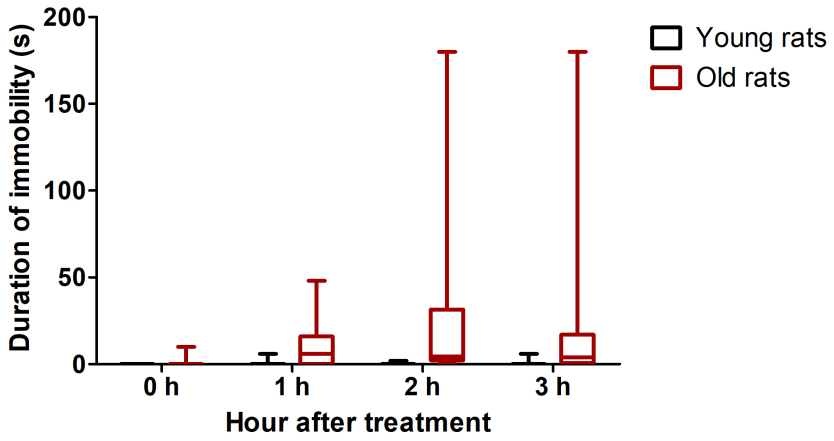

Figure 5. Effect of KCl 500 mg/kg on the duration of immobility response induced by bandaging in young and old rats. The boxes and whiskers represent the median plus the maximum and the minimum value. Two-way RM ANOVA.

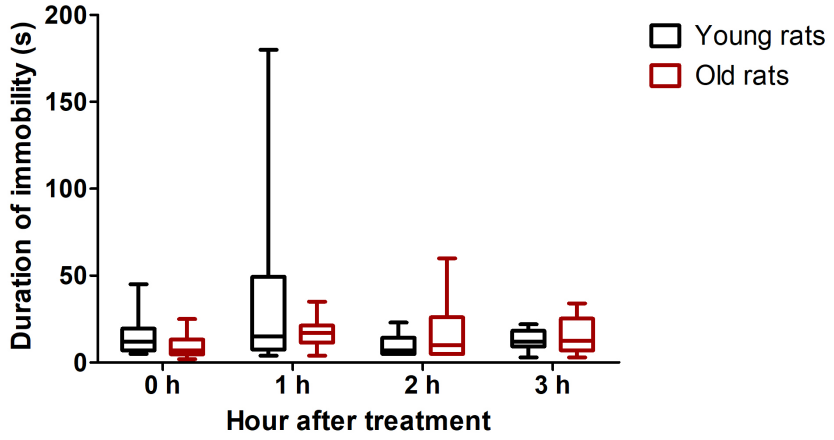

Figure 6. Effect of KCl 500 mg/kg on the duration of immobility response induced by grasping in young and old rats. The boxes and whiskers represent the median plus the maximum and the minimum value. Two-way RM ANOVA.

4. Discussion

The results show that the rat exhibited distinct degrees of susceptibility to induction of IR by the four methods employed. After saline injection, IR was induced by clamping and grasping, but not by inversion or bandaging. The administration of KCI or spironolactone significantly potentiated the IR duration by clamping but not by grasping and significantly potentiated the occurrence and the IR duration induced by bandaging but not by inversion. These data suggest that the most potent methods to induce immobility are clamping and grasping, followed by bandaging and finally by inversion. Both clamping and grasping involve a powerful tactile stimulus to the nape of the neck, which is sustained until

The animal returns to the prone position (clamping) or it emits an escape-like behavior (grasping). The localized application of pressure on the skin of the nape area by both of these methods could be mimicking the mother's biting when carrying the pup or the predator's bite when attacking the prey. The bandaging procedure is similar, because the pressure is applied to an area that includes the neck, but it is different in that the bandage gives a weaker, uniform pressure around the widest area of the neck. This method may elicit some of the components of clamping-induced immobility, because it stimulates the same body area. However, it was shown that bandaging induces IR in neonates, but not in adult rats [11]. The inversion method differs more radically, because there is not a continuous stimulus on the neck during the entire period of immobility. In predator/prey confrontations in nature, the immobility of the prey decreases when the physical distance between predator and prey increase, thus permitting the prey to take advantage of opportunities to escape. However, when the predator and the prey are in a very close contact, the immobility should be potentiated. This could explain why clamping and grasping are more potent to induce the immobility response compared to bandaging and simple inversion. However, bandaging produces a weak stimulus, it still was able to produce immobility, but this was not the case with the simple inversion.

A previous study [11] demonstrates that immobility in neonate rats can be induced either by clamping or by bandaging the neck region, whereas in adult rats it can be elicited only by clamping. Therefore, we concluded that in the adult rats, the characteristics of the stimuli to induce immobility are more specific, and that such specificity is lost in mesencephalic rats [7].

Several lines of evidence indicate that the neocortex is involved in inhibiting the IR response: 1) this susceptibility decreases in the young animals at the time when the neocortex matures [45]; 2) the surgical decortication of unsusceptible animals can make them susceptible [45]; 3) KCl-induced spreading depression of the neocortex also potentiates the IR [46] [47]; and 4) mesencephalic rats are more susceptible to IR than intact adult rats [48]. The mesencephalic rats are similar to neonatal rats, but have more diverse stimuli, either clamping or bandaging are capable of inducing IR.

Aging of the nervous system is critical, because all other body systems are coordinated by this system. The nervous system controls body functioning through the nerve fibers in the voluntary muscles, through the autonomic nervous system or through the signals of the brain to the endocrine system. The decline in the ability of the body to respond to stress is primarily the result of aging in the autonomic nervous system. This system regulates the internal environment of the body. It controls and regulates activities such as heart rate, blood pressure, skin temperature, digestion, elimination, respiration, and reactions to emergencies or stress.

With aging, many of the responses controlled by the autonomic nervous system become slow or weaker (including the neocortical activity) [49], so it takes longer for the body to adapt to changing conditions. This slowdown may be the

result of changes in the synthesis of neurotransmitters, to the structural changes in neurons, or to the loss of neurons [49].

The aging autonomic nervous system manages to maintain normal body functions fairly well when no demands are placed on it, but it falters when it is faced with extreme environmental changes.

Old, neonatal and mesencephalic rats respond in a similar way to more diverse stimuli, involving either clamping or bandaging [48]. Therefore, the evidence seems to provide some basis for formulating the hypothesis that the neocortex could be "weakened" in old rats by the administration of potassium, at least for some IRs (clamping and bandaging).

These data suggest that IR and changes in the potassium serum levels are somehow related. Consequently, such a relationship may be a suitable model to study some forms of paralysis in human beings (i.e. the "familiar periodic paralysis"), which are essayed in transgenic animal models related to changes in the potassium serum levels through changes in the expression of ionic channels [50].

The effects of spironolactone 75 mg/kg were mainly on the immobility induced by clamping (**Figure 1**) and the immobility induced by grasping (**Figure 3**), but it had no effect either on the immobility by bandaging (**Figure 2**) or by inversion (Data not shown). Spironolactone on clamping had its effect at the first hour after administration and it remained until the third hour, for grasping the effect was only significant during the second hour after administration.

On the other hand, KCl provoked an increase in the duration of immobility on three (clamping, bandaging and grasping) of the four kinds of immobility studied. Nevertheless, there was a differential effect due to the duration of immobility by clamping and by bandaging increased only by the highest dose tested (KCl 500 mg/kg), contrarily the duration of immobility by grasping only was augmented by the lowest dose of KCl (300 mg/kg). It should be noted that the KCl at the highest dose (500 mg/kg) maintained its effect until the third hour after administration, whereas the KCl at 300 mg/kg had its greatest effect in the first hour after its administration, and then the effect was lost.

These results may reflect the relationship between serum potassium levels and the duration of the immobility responses studied. The spironolactone that is related to the increase of potassium in serum, as well as the KCl administered directly, increased the duration of the immobility responses that we studied. However, as already mentioned before, these immobility responses show differences in the stimuli that trigger them. We can clearly see this in how its duration was modified differently with changes in serum potassium. Thus, KCl 500 mg/kg increased the duration of clamping and bandaging, but not of grasping. Grasping did increase its duration, but only with the lowest KCl dose (300 mg/kg). It is possible that spironolactone increased potassium levels without becoming as high as KCl 500 mg/kg. We can infer this, since spironolactone did not significantly modify the duration of immobility by bandaging, but it did change the duration of clamping and grasping. Bandaging was only modified with the high-

est KCl dose (500 mg/kg). On the other hand, the duration of grasping was only modified with the lowest dose of KCl (300 mg/kg) and spironolactone, and the duration of immobility by clamping was only increased with the highest dose of KCl (500 mg/kg) and spironolactone. For all this, it is possible that spironolactone has increased potassium levels in the rat to concentrations between 300 and 500 mg/kg.

Spironolactone is an antagonist of aldosterone (hormone involved in the regulation of sodium levels), which is released in the adrenal glands. However, due to its chemical structure, it could also have effects on other hormones released in these glands. For example, it could interfere with the activity of cortisol. In a previous study, our group found that the IR (especially clamping) and the function of the glucocorticoids such as corticosterone are in somehow related, due to a longer IR when corticosterone is administrated [51]. We also found in a model of autism in rats, that these rats had longer immobility compared to their controls. This suggests that rats subjected to this model are prone to more stress and therefore release more cortisol [52].

Comparing the results with symptoms of Hyper PP or Hypo PP, we found that high levels of potassium increase the duration of IR. In addition, we observed stiffness in immobility by clamping and by grasping, which can be similar to the effects in patients with Hyper PP, but bandaging increased their duration of immobility with KCl 500 mg/Kg. However, these changes were not long-term because these rats were normokalemics, and the increase of potassium levels were returned to basal levels. The same occurred with low levels of potassium. When IR were tested at high doses of potassium or when the potassium levels were decreased through increasing the sodium levels, it showed a tendency to increase the duration of IR, that could be due to a paradoxal depolarization [24] [25] [53] [54] or another unknown mechanism.

On the other hand, KCl at high doses (500 mg/kg) showed more susceptibility in old rats to modify the duration of IR by clamping than young rats. Although immobility by bandaging increased its frequency of occurrence, it did not become significantly different from young rats. Grasping did not present differences between old and young rats. However, it is important to remember that immobility by grasping was only modified at low doses of potassium. So, it might be convenient for future studies to test KCl at low doses in young and old rats. In some cases, the presence of Hypo PP and Hyper PP in the patients occurs around the first decade and according to the age of the patient the paralysis are less frequent [17] [18], though it has been observed that old rats showed a major increase in the IR.

In this work we found a clear relation between high levels of potassium and the increase of the duration of immobility responses, specially clamping, bandaging and grasping. However, the effect was not exactly the same, the high levels of potassium modify the duration of these kinds of immobility differentially. As we explained before, the immobility responses can be affected by drugs, age,

Table 1. Summary of the differential effect of the IR elicited by four immobility responses in old rats.

	Immobility			
	Inversion	Clamping	Bandaging	Grasping
Undrugged	–	+	–	+
Potassium and/or spironolactone	–	++	+	++

– absence of response, + presence of response, ++ response enhanced. **Table 1** shows a summary of the differential effect of the IR elicited by inversion, clamping, bandaging, and grasping. When undrugged, only clamping and grasping but not inversion and bandaging induced IR in rats. Spironolactone and potassium significantly enhanced the duration of the IR by clamping and by grasping, and only potassium significantly increased the occurrence and duration of the IR by bandaging but not by inversion. Since there was no effect in the duration of the IR by inversion in animals treated with any drug, these results are not shown.

stress and even some animal models for autism, etc. [2] [3] [11] [51] [52]. Nevertheless, it had not been studied the participation of the electrolytes on the immobility responses, although it is known that an imbalance in electrolytes induce some types of paralysis such as Hypo PP and Hyper. This opens the question about how the hormones, the neurotransmitters and the electrolytes are related to affect the immobility responses in a different way.

5. Conclusion

To summarize, the IR which were modified by KCl were clamping, bandaging and grasping (as shown in **Table 1**) whereas the changes with spironolactone in these kind of IR were only in clamping and grasping. These effects showed that IR can be modified for changes, in this case the increase, in the levels of potassium induced by the direct administration of potassium or by administration of substances such as spironolactone that can alter the homeostasis of potassium. This could lead us to consider the immobility responses as a test to study the Hyper PP or Hypo PP.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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