**Need-induced sodium appetite in SHR is enhanced after one episode of water deprivation-partial repletion (WD-PR) and enhancement in sodium appetite is delayed after multiples episodes**

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SHR have an increased need-free ingestion of sodium solutions due to high cRAS activity, which mediates WD-PR-induced $Na^+$ appetite in HTZ rats. The objectives were to investigate $Na^+$ appetite in SHR submitted to one WD-PR and $Na^+$ appetite enhancement in SHR treated repeatedly. Adult male SHR, WKY and HTZ ($n = 6–12$) were either not deprived, one or multiple WD-PR, and all underwent respective SAT. After 26 h WD-PR, SHR ingested (ml) more 1.8% NaCl ($4.5 \pm 0.2$) than WKY ($0.7 \pm 0.2$) and HTZ ($1.2 \pm 0.5$). Right before access to 1.8% NaCl, Fos-ir (positive nuclei/$10^{-3}$ $mm^2$) was enhanced in SHR compared to both WKY and HTZ in the SFO ($11.0 \pm 3.0$, $6.5 \pm 1.1$, $5.0 \pm 1.3$, respectively), pre-LC ($4.8 \pm 1.4$, $1.7 \pm 0.4$, $1.9 \pm 0.6$) and comNTS ($3.0 \pm 0.7$, $0.9 \pm 0.3$, $1.2 \pm 0.4$). During multiple WD-PR, the 1.8% NaCl intake (ml) enhanced in all subsequent SAT when compared to the 1st in WKY ($1.4 \pm 0.2$, $1.2 \pm 0.1$, $1.6 \pm 0.3$, 2nd to 4th, respectively) and in HTZ ($3.6 \pm 0.6$, $3.1 \pm 0.4$, $2.2 \pm 0.7$). Only the last SAT showed enhancement in SHR ($4.5 \pm 0.2$, $4.2 \pm 0.2$, $5.5 \pm 0.2$). SHR showed higher $Na^+$ intake than normotensive strains in both SAT and the non-deprived state. The increased Fos-ir in the SFO, pre-LC, and comNTS suggest that these areas may trigger and/or drive the enhanced $Na^+$ appetite exhibited by SHR. Moreover, the SHR's delayed enhancement of $Na^+$ appetite facing repeated episodes of WD-PR could be due to their high cRAS activity.

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**Hypothalamic gene expression and angiotensin receptor (AGTR) type-1 protein from different brain areas in hydrated animals submitted to a single or multiple water deprivation-partial repletion (WD-PR)**

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Repeated episodes of WD-PR enhance need-free and induced $Na^+$ intake. The cRAS mediates WD-PR induced $Na^+$ appetite, but the central mechanisms involved in the enhancement of need-free $Na^+$ intake are not well established. We investigate hypothalamic gene expression and cAgtr1 protein in hydrated animals submitted to one or repeated WD-PR. Adult male age-matched SD rats (3–9) were either not deprived or subjected to 1 or 3 WD-PR, and sacrificed in a hydrated status. In one set, the whole hypothalamus was removed for microarray study validated by qRT-PCR. The analysis included Crh, Agtr1a, Syt9 and Oxtr. In a second set, total protein from the OVLT, SFO, PVN, SON, and AMY's brain punches was subjected to Western-blot for Agtr1 expression. Densitometric measurements of the ir bands for Agtr1 were normalized using β-actin. Hypothalamic gene expression of Agtr1a was increased by 76% after a single WD-PR compared to control group. Gene expression of hypothalamic Crh, Syt9, and Oxtr were not altered within groups. Agtr1 protein expression was decreased by 45% in the SFO after both 1 and 3 WD-PR, but no changes were observed in OVLT, PVN, SON, and AMY. In conclusion, the neural substrate for the enhancement in the need-free sodium intake might not be the same as in the substrate for induced sodium appetite.

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**Oxytocin enhances glutamatergic afferent transmission and produces an inward current in second-order medial solitary tract neurons**

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Vagal afferents contact neurons within the NTS and evoke homeostatic reflex pathways. Descending projections from the PVN release oxytocin to modulate afferent communication with NTS neurons, however, the mechanisms through which oxytocin acts are poorly understood. From male Sprague–Dawley rats, horizontal brainstem slices containing the solitary tract (ST) and medial NTS were isolated under deep isoflurane anesthesia. Brain slices were maintained in physiological bath and whole-cell patch clamp recordings were made under voltage clamp. Remote electrical shocks to the ST produced highly consistent glutamatergic EPSCs and identified NTS neurons receiving direct ST afferent innervation. Oxytocin increased the amplitude of ST-evoked EPSCs with no effect on event kinetics. Variance-mean analysis of ST-evoked EPSCs indicates oxytocin increases the release probability of glutamate from the afferent terminals suggestive of a presynaptic site of action. Glutamate mediated miniature EPSCs (mEPSCs) were isolated with TTX and gabazine. Oxytocin increased the frequency of mEPSCs but had no effect on the event kinetics. The oxytocin receptor antagonist blocked this effect on mEPSCs. In addition, oxytocin (1000 nM) evoked an inward holding current consistent with postsynaptic modulation of ion channels. Taken together these findings suggest oxytocin released from PVN projections may enhance glutamatergic vagal afferent transmission within NTS via both pre- and post-synaptic sites of action.

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**The central signaling pathways of amylin: A neuroanatomical study**

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Peripheral amylin inhibits food intake via activation of the area postrema (AP). This activation is synaptically transmitted to the nucleus of the solitary tract (NTS), lateral parabrachial nucleus (LPB), central amygdaloid nucleus (Ce) and bed nucleus of stria terminalis (BST). Interestingly, neurons of the lateral hypothalamic area (LHA), which are activated during fasting, are indirectly inhibited by peripheral amylin. Using a retrograde tracer (cholera toxin B, Ctb) we analyzed whether the LHA receives neuronal projections from amylin-activated brain areas. The anterograde tracer biotinylated dextran amine (BDA) was used to confirm the projections. After Ctb injection into the LHA, 33% of amylin-activated (c-Fos positive) neurons in the LPB projected to the LHA. Only 9% and 7% of amylin-activated neurons from the NTS and BST, respectively, projected to the LHA, while no projections from the AP and Ce were found. Anterograde tracing produced consistent results. Besides ascending projections within the AP–BST axis, we also identified dense projections from the LPB to the ventromedial and paraventricular nuclei. The LPB appears to be the main relay for amylin signal from the AP/NTS region to the LHA, mediating the amylin-induced inhibition of LHA. In addition to conveying excitatory input from the AP/NTS to the Ce and BST, the amylin-activated LPB area also projects to other hypothalamic feeding centers that may be involved in amylin's anorectic action. Detailed knowledge of amylin's mode of action is important for its clinical use in obesity treatment.

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