The nature of non-linear interaction between P2 purinergic and α_1 adrenergic receptors in hypoglossal motoneurons is determined by the temporal pattern of receptor activation

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Hypoglossal motoneurons (XII MNs) innervate the genioglossus muscle of the tongue and increase muscle tone during inspiration, thereby protecting airway patency. Their activity is constantly adjusted by a vast number of neuromodulatory systems to meet the changing demands placed on the respiratory system such as those accompanying changes in behaviour (suckling, vocalisation), environment (hypoxia) and arousal state (sleep-wake cycling). Reductions in XII MN activity and genioglossus muscle tone during sleep are believed to contribute to obstructive sleep apnea in adults and sudden infant death syndrome in newborns. Thus, there is considerable interest in understanding how modulatory systems alter the activity of XII MNs, particularly during sleep. Norepinephine (NE) potentiates XII MN activity primarily through activation of α_1 adrenergic receptors and subsequent blockade of a resting K^+ conductance. Reduced release of NE during sleep is believed to contribute to a reduction of XII MN activity, airway instability and apnea. Extracellular adenosine 5'-triphosphate (ATP) also potentiates inspiratory XII motor output through activation of P2 receptors. A likely source of ATP is via co-release with NE. The goal of this study was to explore how NE and ATP signaling systems interact to affect XII MN activity and inspiratory motor output. Phenylephrine (PE, α_1 adrenergic receptor agonist, 1-100 μM) and ATP (0.1-10 mM) were applied alone and together (in the presence of 100 μM theophylline), either simultaneously or sequentially, to the XII nucleus of medullary slice preparations that continue to generate a respiratory-related rhythm *in vitro* following isolation from anaesthetised neonatal rats.

Bath or local application (15-30s) of PE or ATP alone potentiated ipsilateral XII inspiratory nerve output (n=6), and activated inward currents in whole-cell voltage-clamped XII MNs (n=90). PE responses developed slowly and lasted for many minutes. ATP responses comprised a rapid-onset excitatory component, presumably mediated by P2X receptors. These fast, ATP-gated inward currents were further classified according to their desensitisation kinetics as fast-, slow- and non-desensitising. The excitatory phase was followed in some cases by a slow onset, inhibitory component which manifest as a decrease in burst amplitude or a small amplitude outward current, suggestive of a P2Y receptor mechanism. To assess interactions, we compared the magnitude of currents induced by PE and ATP alone and in combination. ATP, when applied prior to PE, attenuated the PE current to 62% of control (n=32; $p<0.01$), suggesting a negative interaction. Surprisingly, when PE was applied prior to ATP, a positive interaction was observed. In 45% of MNs (16 of 35), particularly those showing non-desensitising responses to ATP, PE caused up to a 3-fold potentiation of both the fast-inward and slow-outward components of the ATP current. That this did not simply reflect differences between MNs was demonstrated in 5 MNs (in 1 μM TTX) where both positive and negative interactions were produced by switching the order of agonist application.

In summary, we have defined a novel mechanism where the nature of the non-linear interaction (positive vs. negative) between two neuromodulatory signaling cascades is determined by their temporal pattern of activation. While the underlying pathways remain to be determined, it is clear that such a mechanism will dramatically increase the dynamic range over which a given modulatory input can modify neuronal excitability.

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