Supplementary Note

The degree of chemosensitivity of midbrain raphe neurons was equivalent to that previously reported for medullary raphe neurons. Chemosensitivity of cultured midbrain raphe neurons was quantified under identical conditions and using the same three methods of analysis previously used for medullary raphe neurons\(^3\): 1) Percentage increase in firing rate from control levels, 2) Absolute firing rates at pH 7.4 and at pH 7.18, and 3) Change in firing rate. All three methods were used, because it is not clear which form of analysis is most relevant to the effect of these neurons on downstream neurons \textit{in vivo}. In response to hypercapnic acidosis, the firing rate of midbrain acidosis–stimulated neurons increased from 0.95 ± 0.19 Hz to 1.97 ± 0.24 Hz (mean ± SEM). In comparison, medullary acidosis–stimulated neurons increased their firing rate from 1.07 ± 0.18 to 2.45 ± 0.22. The firing rate of midbrain acidosis–stimulated neurons increased to 405% ± 70% of control, compared to 285% ± 46% of control for medullary acidosis–stimulated neurons.

Of 13 acidosis–stimulated neurons that were recovered after immunohistochemistry, nine were TPH immunoreactive. Thus, the majority of acidosis–stimulated neurons from this region were serotonergic. However, the existence of some exceptions is different than our previous results from cultured medullary neurons, in which all acidosis–stimulated neurons were serotonergic\(^2\). It is possible that there is a nonserotonergic population of midbrain neurons that is intrinsically chemosensitive. However, a recording was made in low–calcium / high–magnesium Ringer (20 mM MgCl\(_2\) and 0.2 mM CaCl\(_2\)) from one of these four neurons and the response to acidosis was blocked. In contrast, the response of one acidosis–stimulated serotonergic neuron was not blocked by low–calcium / high–magnesium Ringer. Thus, it is more likely that these nonserotonergic neurons were not intrinsically chemosensitive, but were instead stimulated by synaptic input from serotonin neurons.

The percentage of acidosis–stimulated and acidosis–inhibited neurons was different in midbrain cultures and midbrain slices. The most likely explanation for this is that tissue obtained for culture was from a slightly different region than that targeted during slice recordings. In contrast to acidosis–stimulated neurons, none of the 10 acidosis–inhibited neurons from midbrain cultures exhibited the regular firing pattern and interspike ramp.
depolarization characteristic of serotonin neurons. As previously described for acidosis–inhibited neurons cultured from the medulla\textsuperscript{1}, these acidosis–inhibited neurons had smaller somata than acidosis–stimulated neurons, usually with a fusiform soma. Five acidosis–inhibited neurons were recovered after immunohistochemistry, and none were TPH immunoreactive.

Supplementary Reference List


