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COMMUNICATIONS

Buspirone decreases the activity of 5-hydroxytryptamine-containing dorsal raphe neurons in-vitro

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Buspirone, a non-benzodiazepine anxiolytic agent, produced a dose-dependent decrease in the activity of 5-HT-containing dorsal raphe neurons recorded from mouse brain slices. The response was not changed in a low calcium/high magnesium incubation medium, indicating that the observed effects were the result of a direct action of buspirone on raphe neurons. These data are discussed within the context of the anxiolytic effects of buspirone.

Buspirone is a novel anxiolytic agent that has attracted a great deal of scientific interest recently due to its potential clinical usefulness (Goldberg & Finnerty 1979). Recent studies have shown that, in contrast to the benzodiazepine anxiolytics, buspirone increases the firing rate of noradrenaline (NA)-containing locus coeruleus neurons in anaesthetized rats (Sanghera et al 1983). Furthermore, we recently demonstrated that buspirone also produces an excitation of NA neurons when recorded from brain slices (Trulson & Henderson 1984). These studies suggest that NA neurons may not be as important in mediating anxiety states as previously suggested.

We recently demonstrated that buspirone produces a dose-dependent decrease in the activity of 5-HT-containing dorsal raphe (RD) neurons in unrestrained cats (unpublished data). However, using the in-vivo preparation, it is not possible to determine whether the effects of buspirone are direct effects on raphe neurons, or whether the drug alters some afferent input to the RD nucleus. In the present study we examined the effects of buspirone on the activity of 5-HT-containing RD neurons recorded from mouse brain slices, a preparation that severs virtually all afferent inputs to the RD.

Method

Adult, male, Swiss Webster mice (18–23 g) were decapitated. The brains were rapidly removed and cut into 400 μm sections using a McIlwain tissue chopper. Two slices containing the RD nucleus from each brain were incubated in a recording chamber in standard Yamamoto's solution (1973) for 1 h at 35 °C under a moist atmosphere saturated with 95% O_2–5% CO_2 before the initiation of recording. The composition of the incubation medium was as follows: Na^+, 150; K^+, 6·24; Cl^-, 131; Ca^{2+}, 2·4; Mg^{2+}, 1·3; PO_4^{3-}, 1·24; SO_4^{2-}, 1·3; HCO_3^-, 26; and D-glucose, 10 mM. The total incubation volume was 2·0 ml. Single units were recorded using micropipettes with tip diameters of 2–3 μM and impedances ranging from 2·2–3·0 MΩ. The micropipettes were filled with 2 M NaCl saturated with Fast Green dye. Electrodes were lowered into the RD with a hydraulic microdrive with the aid of a dissecting microscope. Extracellular unit activity was amplified (Grass P511 preamps), filtered (Bandpass 0·5–3·0 kHz), monitored on a storage oscilloscope and audiomonitor, and stored on magnetic tape. Discharge rates were obtained on-line using an electronic counter triggered by the standard pulse output of a Schmitt trigger.

5-HT-containing RD neurons were identified on-line by their slow, regular discharge rate, which has been shown to be characteristic of these neurons in anaesthetized rats (Aghajanian & Haigler 1974), freely moving cats (Trulson & Jacobs 1979), and in mouse brain slices (Trulson et al 1982a). In a subgroup of cells, the 5-HT nature of the neurons was further confirmed by the characteristic depression of unit activity following iontophoretic application of L-tryptophan and lysergic acid diethylamide (LSD) at low ejection currents (Aghajanian & Haigler 1974).

After baseline unit activity had been obtained, buspirone was added to the incubation medium by microsyringe in a final concentration of 0·5, 1·0, 2·5, 5·0, or 10·0 μM, and unit activity was recorded for 40–60 min. In an additional set of experiments, the effects of the same concentrations of buspirone on RD unit activity were examined in modified Yamamoto's solution containing 0·5 mM Ca^{2+} and 10 mM Mg^{2+}, a procedure known to block all synaptic transmission (Rubin 1970). Only one concentration of buspirone was tested on each slice. The data were analysed by obtaining the mean discharge rate for each cell during 5 min on baseline and 5 mins post-drug (30–35 min). The statistical analysis consisted of a two-way analysis of variance (ANOVA) (type of incubation of medium and dose, using no-drug, i.e. baseline condition, as a dose of 0), followed by Newman-Keul's tests for differences between baseline condition and each of the drug doses. The ED50 value was determined by plotting the mean percent inhibition for each dose on log-probit paper, as

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described by Litchfield & Wilcoxon (1949), and finding the 50% inhibition point on the best fit straight line determined by the least squares method.

At the end of each experiment, Fast Green dye was deposited by passing current through the recording electrode. The recording sites were then determined using the histological method of Trulson et al (1984).

Results

5-HT-containing neurons in the RD displayed a spontaneous discharge rate of 2 to 6 spikes s^{-1} (mean = 3·19 spikes s^{-1} in standard Yamamoto's solution; mean = 3·22 spikes s^{-1} in low Ca^{2+}/high Mg^{2+} medium). Analysis of variance (ANOVA) revealed no significant differences between the overall discharge rates in standard versus altered Yamamoto's solution ($P > 0·2$). Buspirone produced a dose-dependent decrease in RD unit activity ($P < 0·01$, ANOVA). The ED50 values were 2·2 and 2·6 μM for standard and low Ca^{2+}/high Mg^{2+} media, respectively. Unit activity was depressed within 5–10 min after adding buspirone to the incubation medium, and the change in activity persisted for more than 30 min. The lowest concentration of buspirone (0·5 μM) produced a small, but statistically significant change in RD unit activity from baseline levels (Table 1). RD unit activity was progressively decreased with increasing concentrations of buspirone in the incubation medium, reaching a maximum depression of activity greater than 90% at 10 μM concentrations (Table 1). The change in unit activity was reversed by exchanging the incubation medium for fresh Yamamoto's solution using a non-pulsating exchange pump. Histological analysis revealed that all recording sites were located within the RD, as expected.

Table 1. Effects of buspirone on 5-HT neuronal activity in mouse brain slices. Data are presented as mean spikes s^{-1} ± s.e.m. for the number of cells indicated in parentheses. Percent change values represent change from 0 drug baseline activity. The significance of differences from 0 drug values were evaluated using Newman-Keuls tests: $^{*}P < 0·05$; $^{**}P < 0·01$.

Buspirone concentration (μM)	Incubation medium	
	Standard	High Mg^{2+}/ low Ca^{2+}
0	3·19 ± 0·15	3·22 ± 0·16
—	—	—
(n)	(29)	(27)
0.5	2·84 ± 0·1*	2.96 ± 0.14
% change	−11·0%	−8·1%
(n)	(33)	(38)
1·0	1·95 ± 0·23**	2·13 ± 0·21*
% change	−38·9%	−33·9%
(n)	(20)	(22)
2·5	1·02 ± 0·14**	1·34 ± 0·24**
% change	−68·0%	−58·4%
(n)	(17)	(19)
5·0	0·26 ± 0·08**	0·53 ± 0·11**
% change	−91·8%	−83·5%
(n)	(14)	(12)
10	0·11 ± 0·02**	0·17 ± 0·05**
% change	−96·6%	−96·7%
(n)	(6)	(5)

Discussion

The present study is apparently the first to demonstrate that buspirone suppresses the activity of 5-HT-containing RD neurons in-vitro. Furthermore, the fact that this effect occurred in low Ca^{2+}/high Mg^{2+} medium, a procedure known to block all synaptic transmission (Rubin 1970), suggests this is a direct effect of buspirone on raphe neurons and not the result of some change in the afferent input to the RD. These data also suggest that buspirone itself, rather than some metabolite of the drug, is responsible for the observed effects. The finding that buspirone decreases the activity of 5-HT-containing RD units is consistent with previous studies which have shown that buspirone decreases the levels of the major metabolite of 5-HT, 5-hydroxyindoleacetic acid, in the limbic forebrain (Hjorth & Carlsson 1982).

Previous studies have demonstrated that buspirone *increases* the discharge rate of noradrenergic neurons in the locus coeruleus (Sanghera et al 1984; Trulson & Henderson 1984). Buspirone has also been shown to increase the firing rate of dopaminergic neurons in the substantia nigra (McMillen et al 1983).

Both buspirone and the benzodiazepine anxiolytics suppress the discharge rate of RD neurons in-vitro. However, when examined in animals in-vivo, benzodiazepines have been reported to produce inhibition of raphe unit activity only at very high doses (Trulson et al 1982b). Buspirone, on the other hand, suppresses the activity of 5-HT-containing neurons at doses commonly used for its "anxiolytic" properties in animals (unpublished data). Buspirone does not possess the sedative-hypnotic, muscle relaxant and anticonvulsant properties characteristic of the benzodiazepines (Riblet et al 1980). Previous studies from our laboratory have suggested that at least some of these latter properties of benzodiazepines may be attributable to suppression of activity of dopamine-containing substantia nigra neurons (Trulson 1984). Buspirone, however, has been demonstrated to produce the opposite effect on dopaminergic neurons (McMillen et al 1983). Therefore, a common effect of buspirone and benzodiazepines is their ability to suppress the activity of 5-HT-containing neurons. However, as pointed out above, the benzodiazepines produce this effect only at doses well above those commonly used for their anxiolytic properties.

It is not known what the underlying cellular mechanism by which buspirone suppresses the activity of RD neurons might be. There is evidence that it acts as an agonist at 5-HT autoreceptors on the cell bodies and/or dendrites of these neurons (Glaser & Traber 1983; Riblet et al 1984). Buspirone binds with high affinity to 5-HT-S_1 receptors in the brain of rats (Glaser & Traber 1983; Riblet et al 1984). Furthermore, it has been

demonstrated that 5-HT-S_1 autoreceptors on nerve terminals in the cerebral cortex are similar to the pre-synaptic 5-HT-S_1 receptor (Middlemiss 1984).

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Comparative lipophilicities of substrates of monoamine oxidase

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Oil/water partition coefficients of various substrates of monoamine oxidase (MAO) and kinetic parameters of MAO-A and -B of rat liver at two pH values, pH 7 and pH 9, were investigated. Octanol, heptane or benzene were chosen for the oil phases. The deamination of the biogenic amines 5-hydroxytryptamine (5-HT), tyramine, 2-phenethylamine (PEA) and benzylamine was studied at pH 7 and pH 9. Results indicated all four substrates were very hydrophilic, and the oil/water partition coefficients of benzylamine and PEA were higher than those of 5-HT and tyramine. The changes in K_m and V_{max} values at pH 7 and pH 9 indicated that the affinities of MAO-A towards 5-HT and tyramine slightly increased at pH 9 and those of MAO-B towards tyramine and benzylamine also increased at pH 9, while uncharged amines at pH 9 amounted to about a hundred times of those at pH 7. It is concluded that the mitochondrial MAO bound to the membrane may metabolize charged molecules as well as uncharged counterparts.

Monoamine oxidase (monoamine; oxidoreductase deaminating EC 1.4.3.4, MAO) catalyses the oxidative deamination of different monoamines, and is assumed to exist in two forms, MAO-A and MAO-B. This is based primarily upon its substrate specificity and inhibitor sensitivity (Johnston 1968; Houslay & Tipton 1974; Houslay et al 1976; Knoll 1976). MAO-A is more readily inhibited by clorgyline and appears to have a preference for 5-HT and noradrenaline, while MAO-B is more sensitive to selegiline (deprenyl) with its preferred substrate being 2-phenethylamine (PEA) and benzylamine. Other amines, such as tyramine, tryptamine and dopamine, are thought to be substrates acceptable to both enzyme types.

There is considerable experimental support for the existence of two different protein species (Callingham & Parkinson 1979; Cawthon & Breakefield 1979). However, the hypothesis that the enzyme exists in a single form which is modified by its specific membrane environment is also supported by many observations (Houslay & Tipton 1973, 1974; Tipton et al 1976). There is evidence that substrate specificity may be affected differently by the lipid environment (Houslay 1980).

To provide further information about the basic interactions that govern the substrate specificities of the mitochondrial monoamine oxidase of rat liver, the present paper reports studies of the oil/water partition coefficients of substrates and the effect of pH on the kinetic parameters of MAO.

Materials and methods

Oil/water partition coefficients. A 1·0 ml amount of 0·2 M phosphate buffer (pH 7) or 0·2 M carbonate-bicarbonate buffer (pH 10) containing radioactive substrates (0·01–0·10 mM) was mixed with 1·0 ml of one of the organic

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