Ethanol induces bursting amongst electrically coupled snail neurons

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Ethanol has several effects on synaptic transmission and neuronal excitability. It potentiates cholinergic transmission at motor end plates (Gage, 1965; Okada, 1967), and at the motoneuron junctions with Renshaw cells (Meyer-Lohmann, Hagenah & others, 1972) and in sympathetic ganglia (Larrabee & Pasternak, 1952), but depresses excitatory cholinergic transmission in molluscan ganglia (Barker, 1975; Faber & Klee, 1977), and excitatory glutamate-mediated transmission at the crustacean neuromuscular junction (Adams, Gage & Hamill, 1977). In *Aplysia* ganglia ethanol reduces the amplitude of action potentials, may reduce the size of certain excitatory and inhibitory postsynaptic potentials (e.p.s.p.s., i.p.s.p.s.) and may either depolarize or hyperpolarize the resting membrane potential of different neurons (Faber & Klee, 1977). However the observed effects are generally slight (in many cases there is no effect on electrical properties) and are caused by relatively high concentrations of ethanol (4–5%). We have studied the effects of ethanol on a system of electrically coupled neurons in *Planorbis corneus*. The results show that relatively low concentrations of the substance produce marked alterations in the electrical properties of the coupled neurons.

Central ganglia were dissected from *Planorbis corneus* and pinned to the base of a 1 cm^2 recording chamber perfused with physiological saline (Berry, 1972) at room temperature (17–21°). Neurons were impaled with double-barrelled microelectrodes containing 0·6 M K_2SO_4 (about 10 MΩ resistance); one barrel was used for recording potential and the other for passing current. Conventional amplifying and stimulating equipment was used.

In the visceral ganglion there is a group of at least 15 neurons which are electrically coupled to each other. When current pulses of either polarity are passed into any one of these neurons a smaller potential change of similar polarity is produced in the others. A spike in any neuron produces a brief depolarization in the others (Fig. 1). These depolarizations are not significantly reduced by Ca^{2+}-free saline containing 1 mM EGTA and six times the normal Mg^{2+} concentration, which abolishes chemical synaptic potentials (Berry & Pentreath, 1976; Pentreath & Berry, 1977). The neurons receive inhibitory dopaminergic input from a specified dopamine neuron in the left pedal ganglion (Berry & Cottrell, 1975).

The electrically coupled neurons normally fired action potentials spontaneously at a fairly regular rate, but with a tendency to produce couplets of spikes (Fig. 2A). Some fired in synchrony, others independently,

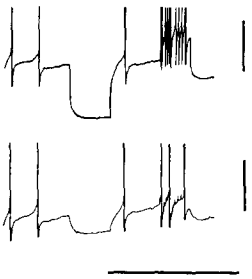

FIG. 1. Intracellular recordings from two of the electrically coupled neurons in the visceral ganglion of *Planorbis corneus*. Hyperpolarizing and depolarizing pulses applied to one of the neurons (upper trace) are transmitted to the other (lower trace). During the spontaneous activity the action potentials were firing in synchrony. When the upper neuron was depolarized the action potentials produced small depolarizing potentials in the coupled neuron. Time scale, 10 s; voltage scales, 25 mV.

depending to some extent on the strength of coupling. Ethanol (0·6–1%) caused a marked change in the firing pattern. Recurrent hyperpolarizing potentials and alternating bursts of action potentials occurred progressively until all the neurons were actively and rhythmically bursting in synchrony (Fig. 2). These effects were produced within 1 min of application, were maintained as long as ethanol was present (tested up to 2 h) and were reversed within 2 to 3 min of washing with pure saline. The threshold concentration of ethanol to cause the effects was about 0·5%. The response was the same in each of 20 preparations studied, irrespective of whether the neurons were initially quiescent or firing spontaneously. It was usually impossible to suppress the bursting by artificially hyperpolarizing one of the neurons. The response to ethanol appeared specific since there was no effect on other, non-coupled neurons. Ethanol (0·5–4%) slightly reduced the dopamine-mediated i.p.s.p.s. elicited in the coupled neurons by stimulation of the specified dopamine neuron. However these effects were difficult to assess because of the large variations in membrane potential of the coupled neurons.

The initiation of bursting by ethanol is in complete contrast to its effects on the endogenously bursting pacemaker neuron R15 in *Aplysia*, where ethanol (2–4%) blocks electrical activity (Faber & Klee, 1977). In *Planorbis*, when the concentration of ethanol was increased (2–4%), this increased the number of spikes per burst and the amplitude of the post-burst hyperpolarization while reducing the amplitude of action

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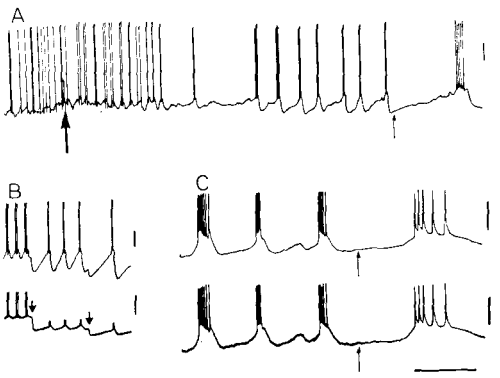

FIG. 2. Induction of bursting activity amongst electrically coupled neurons in *Planorbis* by ethanol. A: Intracellular recording from an electrically coupled neuron which was firing action potentials spontaneously. Action potentials occur frequently in pairs. At arrow (left) 0·8% ethanol was added to the bath, and after a delay of about 40 s burst activity was initiated. For the last burst (right arrow) the speed of recording was increased to demonstrate individual action potentials. Bursting continued for 2 h and activity returned to normal within 3 min of washing. B shows simultaneous recordings from two coupled neurons in a different preparation exposed for 20 min to 0·8% ethanol. The neurons have been induced to fire synchronous bursts of action potentials. At arrows, the neuron recorded in the lower trace was hyperpolarized by current passed through the second barrel of the microelectrode, and the other neuron (upper trace) was indirectly hyperpolarized by current crossing the electrotonic junction between the two neurons. Full action potentials were abolished in the soma of the polarized neuron, but the interburst interval was increased, which indicates that the bursting originates within the network of the coupled neurons. The bursting could not be suppressed by further hyperpolarization. Full spike amplitude was not recorded in the upper trace. C: Simultaneous recordings from coupled neurons in a preparation exposed for 30 min to 0·8% ethanol. The records illustrate the synchronous nature of the induced bursting activity. At the arrows the speed of recording was increased to demonstrate individual action potentials. Time scale: A: 25 s, at right arrow 5 s; B: 25 s; C: 5 s, at arrow 1 s. Voltage calibration: A: 20 mV; B: upper trace 20 mV, lower trace 50 mV; C: both traces 50 mV.

potentials and increasing their duration. Ethanol has no effect on the bursting pacemaker potentials which can be induced by vertebrate peptide hormones in some snail neurosecretory neurons (Barker, 1975). The effects of ethanol on bursting in the coupled neurons are thus opposite to effects described on certain endogenously bursting molluscan neurons.

The bursting elicited by ethanol can be distinguished from that which sometimes occurs spontaneously and which can often be elicited when one of the neurons is depolarized by continuously applied current (Berry & Pentreath, 1977). The latter appears to be caused by regenerative excitation, and synchrony of spikes occurs only at the end of each burst, when the resulting reduction in loading causes prolongation of the spike after-hyperpolarization and termination of the burst (Getting & Willows, 1973, 1974). During bursting induced by ethanol all the neurons fire more or less in synchrony throughout the burst and it is usually impossible to suppress bursting in the network by hyperpolarizing one of the neurons (Fig. 2B, C).

Occasionally, preparations exhibited natural burst activity similar to that induced by ethanol. Thus it appears that ethanol is 'switching on' a process which may occur normally. In neurons showing spontaneous bursting, ethanol increased the amplitude of the post-burst hyperpolarization and the number of spikes per burst. The function of the coupled neurons has not yet been determined, and the possible significance of the bursting is not known.

Several reports have provided evidence that ethanol has a membrane-fluidizing action, which may be associated wth its effects on synaptic transmission and neuronal excitability (Curran & Seeman, 1977; Woodson, Traynor & others, 1977). It is possible that the specialized sites of close membrane apposition which occur at the junctions between electrically coupled neurons (Sotelo, 1975; Pentreath & Berry, 1977) and of which there are increasing numbers of reports of in the cns (Berry & Pentreath, 1976), are also sensitive to such fluidizing effects, and that the initiation of bursting results from ethanol-induced changes at the electrical junctions between the neurons. Although the actual mechanism of action of ethanol remains to be determined, the results show that it induces specific and prolonged regular bursting activity.

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The influence of β-blocking agents on the kinin system in rat plasma

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The hypotensive effect of β-blocking agents has been reported in man (Buhler, Laragh & others, 1972, 1973; Doyle, 1974; Simpson, 1974) and in normotensive or spontaneously hypertensive animals (Roba, Lambelin & Schaepdryver, 1972; Vavra & Greselin, 1973; Lloyd & Nanol, 1975; Sweet & Wenger, 1976) but the mechanism is not clear. An effect on the renin-angiotensin system was postulated and the inhibition of the renin secretion was suggested to be responsible (Weber, Thornell & Stokes, 1974). But according to Rocha e Silva (1963) the renin-angiotensin system can have an antagonistic action towards the kinin system. Therefore we have investigated the influence of β-blocking drugs on the kinin system by estimating in the rat the concentration of kininogen *in vivo* and the activation of prekallikrein *in vitro*.

The β-blocking drugs used were: propranolol (Polfa), alprenolol (Astra), sotalol (Mead. Johnson), Kö 1366—bunitrolol (Boehringer), practolol (Polfa), pindolol—Visken (Sandoz). Phentolamine (Regitine—Ciba), an α-receptor blocking agent was also studied. Male Wistar rats weighing 180–220 g were injected with the β-blocking drugs intraperitoneally. Two h later animals were anaesthetized with ether and blood was collected from the inferior vena cava. The pooled blood from 8 rats was used for the preparation of batches of rat plasma. The concentration of kininogen was estimated according to Briseid, Dyrud & Öie (1970). Kinin was released from kininogen by a plasma and urine kallikrein preparation. The kinin determinations were carried out on the isolated rat uterus as 'bracketing assays' with the standard dose ratio 3:2. Bradykinin (Sandoz) was used as standard.

Activation of prekallikrein *in vitro* was estimated according to Briseid & others (1970) with a small modification. Prekallikrein preparations (0·9 ml) were incubated for 45 min at 37° with propranolol, Kö 1366 or phentolamine added at several concentrations in a constant volume of 0·1 ml. Incubate (0·5 ml) was then added to 0·5 ml of kininogen preparation. After incubation at 37° for exactly 10 min, a 0·5 ml sample was transfered to 2·5 ml of boiling saline. The mixture was boiled for 5 min cooled and its volume was adjusted to 5 ml with saline. The released kinins were assayed as described above.

Table 1. *The influence of β-blocking drugs and phentolamine on the kininogen concentration* (μg ml^{-1} ± s.e.) *in rat plasma.*

Drug	Dose mg kg^{-1}, i.p.	n	Kininogen concn μg ml^{-1} ± s.e.	% decrease
Solvent	—	16	2·00 ± 0·036	—
Propranolol	1	5	1·87 ± 0·08	7
	10	10	1·58 ± 0·048	21**
Phentolamine	5	10	1·99 ± 0·07	—
	10	5	1·99 ± 0·07	—
	15	5	2·00 ± 0·10	—
Solvent	—	13	1·90 ± 0·07	—
Propranolol	20	9	1·50 ± 0·04	25**
Kö 1366	20	7	1·47 ± 0·12	27**
Alprenolol	20	12	1·70 ± 0·05	15*
Sotalol	20	8	1·27 ± 0·06	37**
Pindolol	20	7	1·47 ± 0·12	27**
Practolol	20	17	1·90 ± 0·10	5
	60	5	1·47 ± 0·10	27**

*P <0·01. ** P <0·001.

Propranolol (1 mg kg^{-1}) did not influence the plasma kininogen, concentration but at 10 and 20 mg kg^{-1} it decreased it by about 21 and 25%, respectively. Alprenolol, sotalol, pindolol and Kö 1366 at 20 mg kg^{-1} also reduced the concentration of kininogen in plasma by 15, 37, 27 and 27% respectively. Practolol (20 mg kg^{-1}) appeared without effect while 60 mg kg^{-1} decreased the kininogen concentration by about 27% (Table 1).

Propranolol and Kö 1366, 1×10^{-5}, 1×10^{-4} or 1×10^{-3} g induced dose-dependent activation of prekallikreine. Phentolamine up to 1×10^{-3} g did not produce such an effect.

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