Bicuculline and the Depression of Medullary Reticular Neurones by GABA and Glycine

Bicuculline has recently been reported to be a relatively specific antagonist of the depression by γ-aminobutyric acid (GABA) and synaptic inhibition of neurones in various areas of the mammalian central nervous system[1-3]. In contrast, GODFRAIND, KRNJEVIĆ and PUMAIN[4] were unable to demonstrate an action of bicuculline on the depression produced by GABA of the majority of cerebral cortical neurones. We therefore investigated the action of bicuculline on the depressant effects of GABA and glycine administered microelectrophoretically to neurones of the medullary reticular formation.

Results were obtained from 8 cats which had been decerebrated during nitrous oxide/halothane anaesthesia. The methods have been described previously[5]. Recordings were made from neurones in the reticular formation, 2–5 mm rostral to the obex and up to 2 mm lateral to the midline of the floor of the IVth ventricle. Action potentials were recorded extracellularly through the 4 *M* NaCl-containing barrel of 6-barrel glass micropipettes (4–7 µm tip diameter), and the firing frequency was plotted on an ink recorder. The other barrels contained glycine (Fluka, 0.5 *M*, pH 3–4), GABA (Fluka, 0.5 *M*, pH 3–4), monosodium L-glutamate (Fluka, 0.5 *M*, pH 7.5–8), 165 m*M* NaCl and bicuculline (Pierce Chemical Co., 5 m*M* in 165 m*M* NaCl). To prepare bicuculline solutions, 1.9 mg of the free base was thoroughly ground in 0.1 ml of 0.1 *M* HCl for at least 30 min. During this procedure the solution was periodically heated under warm tap-water (50 °C), and was sometimes left overnight before adding 0.9 ml 165 m*M* NaCl. The pH of the solution was 3. Bicuculline was ejected with a cationic current.

The majority (66%) of the 32 reticular neurones tested were firing spontaneously (20–120 spikes/sec); the remainder were fired by a continuous ejection of glutamate. Bicuculline (30–150 nA) usually had no effect on the firing frequency of the neurones, although in some cases it caused either an increase (4 neurones) or decrease (6 neurones) which was of a slow time course. Barrels containing bicuculline often became 'noisy' with time, especially when high currents (greater than 80 nA) were passed. No obvious differences were observed between results obtained from spontaneously firing and glutamate-fired neurones.

Bicuculline reversibly reduced the depressant action of GABA on 19 of 30 neurones tested. The effects of bicuculline varied between neurones from a slowing of the time course to a complete block of the depression caused by GABA. Typical results are illustrated in the Figure. GABA (20 nA) depressed the spontaneous firing of the neurone by almost 100 spikes per sec whereas Na^+ (100 nA) had little effect. During the ejection of bicuculline (110 nA), the depression caused by GABA (20 nA) was reduced to approximately $^1/_3$ of its initial value, and recovery was complete 30 sec after the current ejecting bicuculline was terminated.

In general, high ejecting currents of bicuculline (usually 3–6 times the GABA currents) were required to affect the depression caused by GABA. The time course of action of bicuculline was usually less than 1 min for both time of onset and recovery. It was not possible to reduce the effects of GABA by bicuculline on about $^1/_3$ of the cells studied, using ejecting currents 2–7 times those used to administer GABA.

Bicuculline was tested on the depressant actions of both GABA and glycine on 16 cells. The currents used to eject the amino acids were selected to produce approximately equal, but just-maximal, effects. Although bicuculline often affected the depression caused by GABA (10 cells), it also reduced the effects of glycine on 6 neurones. In all these cases, depression by GABA was always reduced more than that by glycine. On some neurones glycine depression was reduced only when the currents ejecting bicuculline were markedly increased.

In conclusion, our results indicate that bicuculline often reversibly reduced the depression of medullary reticular neurones by GABA. The depressant action of glycine was sometimes also affected, although to a lesser extent.

Zusammenfassung. Der Einfluss von mikroelektrophoretisch verabreichtem Bicucullin auf die hemmende Wirkung von GABA und Glycin wurde an Neuronen der bulbären Formatio reticularis der nichtnarkotisierten, decerebrierten Katze untersucht. Bicucullin verminderte oder blockierte oft in reversibler Weise die durch GABA hervorgerufene Hemmung. An einigen Neuronen wurde jedoch auch die durch Glycin erzeugte Hemmung geringgradig reduziert.

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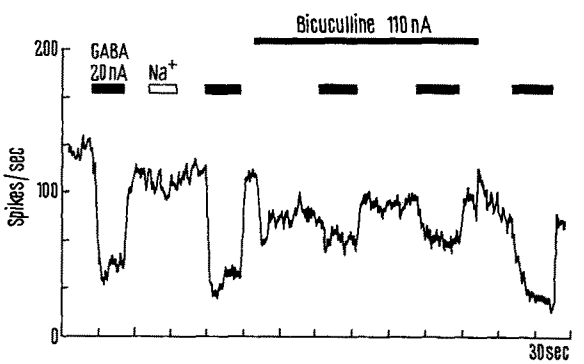

Action of bicuculline on the depression caused by GABA on a spontaneously firing reticular neurone. Firing frequency (ordinate, spikes/sec) is plotted against time (abscissa, 30-sec intervals). Periods of drug ejection are represented by horizontal bars above the trace. All substances were administered with cationic currents. Values of the ejecting currents are given in nA (10^{-9} A). Black thick bars refer to a series of GABA (20 nA) ejections. Na^+ was applied with a current of 100 nA.

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