Amino Acid Neurotransmitters

Advances in Biochemical Psychopharmacology Volume 29

Editors

Francis V. DeFeudis, Ph.D.
Professor of Biochemistry
Université de Strasbourg
Faculté de Médecine
Strasbourg, France

Paul Mandel, M.D., Ph.D.
Professor of Biochemistry
Honorary Director, Centre de Neurochimie du CNRS
Université de Strasbourg
Faculté de Médecine
Strasbourg, France

Raven Press ■ New York

Contents

Amino Acid Uptake and Release

Receptor Pharmacology: Electrophysiological Studies

Amino Acid Receptors: Ligand-Binding and Autoradiographic Studies

Amino Acid Neurotransmitters,
edited by F. V. DeFeudis and P. Mandel,
Raven Press, New York © 1981

4-Aminopyridine Reveals Presynaptic GABA Actions in Rat Sympathetic Ganglia

P. Grafe, M. Galvan, and G. ten Bruggencate

Department of Physiology, University of Munich, 8000 München 2, Federal Republic of Germany

GABA-receptors are present on pre- and postsynaptic membranes in mammalian sympathetic ganglia (1, 3, 4, 5). Recently, we have observed that the axonal K^+-channel blocker 4-aminopyridine (4-AP) selectively enhances presynaptic excitability in isolated rat sympathetic ganglia - a condition which allows a particularly clear demonstration of presynaptic GABA-effects. This interaction of GABA and 4-AP has been used to characterize the presynaptic receptors.

ACTIONS OF 4-AMINOPYRIDINE

Intracellular recordings were made from 35 neurones in rat isolated superior cervical ganglia maintained in flowing Krebs' solution at 25° or 30°C. Drugs were bath-applied.

The most obvious effect of 4-AP on sympathetic neurones was a significant increase in the frequency of spontaneous postsynaptic potentials. Thus, after several minutes superfusion with solutions containing 0.1 - 1 mmol/l 4-AP, 25 out of 36 cells exhibited spontaneous excitatory postsynaptic potentials (EPSPs) as well as action potentials (see fig. 1).

EPSPs and action potentials were reversibly abolished by the nicotinic receptor antagonist hexamethonium (0.5 - 2 mmol/l), indicating that the action potentials resulted from suprathreshold EPSPs. Since there were no marked changes in resting membrane potential and input resistance, we conclude that 4-AP enhances presynaptic excitability in rat sympathetic ganglia leading to spontaneous release of acetylcholine.

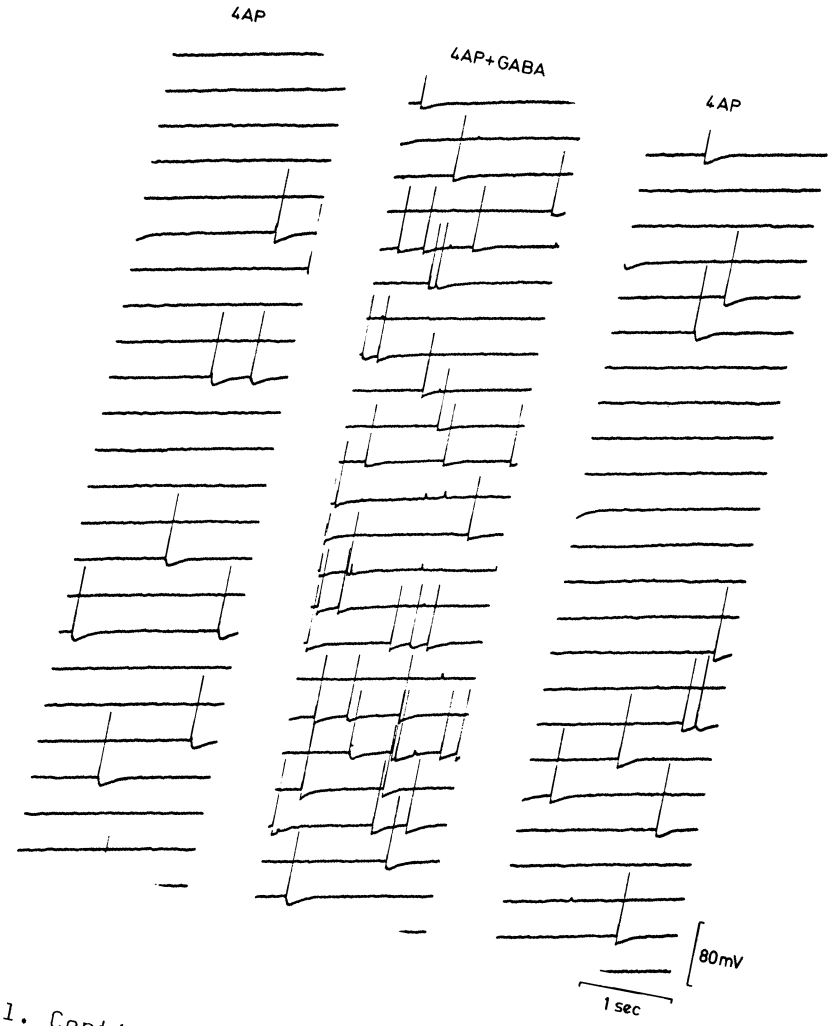

FIG. 1. Continuous registration of the membrane potential (DC-coupled) during intracellular recording from a sympathetic neurone. 4-AP (100 µmol/l) was added 15 min before the recording commenced; the 3 traces show spontaneously occurring spikes and EPSPs before, during and after additional application of 100 µmol/l GABA. Calibration bars 80 mV, 1 sec.

ACTIONS OF GABA ON 4-AMINOPYRIDINE

Figure 1 illustrates the action of 100 µmol/l GABA during a continuous application of 100 µmol/l 4-AP. Under such conditions, GABA clearly increased the frequency of 4-AP induced spontaneous EPSPs; the neuronal depolarization produced by GABA remained unchanged (not illustrated). Hexamethonium also abolished the GABA-induced increases in spontaneous EPSPs/spikes indicating that in the presence of 4-AP, presynaptic actions of GABA are revealed. The question remains as to whether this interaction results from GABA action on nerve terminals (3, 6) or the afferent axonal membrane (3, 4, 6).

5 additional experiments using extracellular recordings with suction electrodes on the postganglionic nerve were performed. This method allowed a simpler analysis of the 4-AP/GABA interaction. Figure 2, for example, illustrates that the frequency of 4-AP induced postsynaptic potentials increased from about 0.25 Hz to 4 Hz in the presence of 100 µmol/l GABA, an effect blocked by hexamethonium.

EFFECTS OF BICUCULLINE AND SOME GABA-MIMETICS

Several experiments, using bicuculline methochloride and GABA-mimetics were performed to characterize the GABA-receptors responsible for the effects described. This seemed interesting in view of the recent suggestion that a second type of bicuculline-insensitive, presynaptic GABA receptor exists in the mammalian peripheral nervous system (2). It was observed that the action of 100 µmol/l GABA in 4-AP was mimicked by 3-aminopropanesulphonic acid (30-50 µmol/l; 9 cells tested) and muscimol (50 µmol/l; 3 cells), but not by baclofen (100-200 µmol/l;8 cells), glutamate, glycine or taurine (all 1 mmol/l; 2 cells). Bicuculline methochloride (30 µmol/l; 4 cells) reversibly antagonised the pre- and postsynaptic actions of GABA.

Therefore, it appears that the GABA receptors responsible for this presynaptic action are similar to those present on the postsynaptic membrane of mammalian peripheral and central neurones (7).

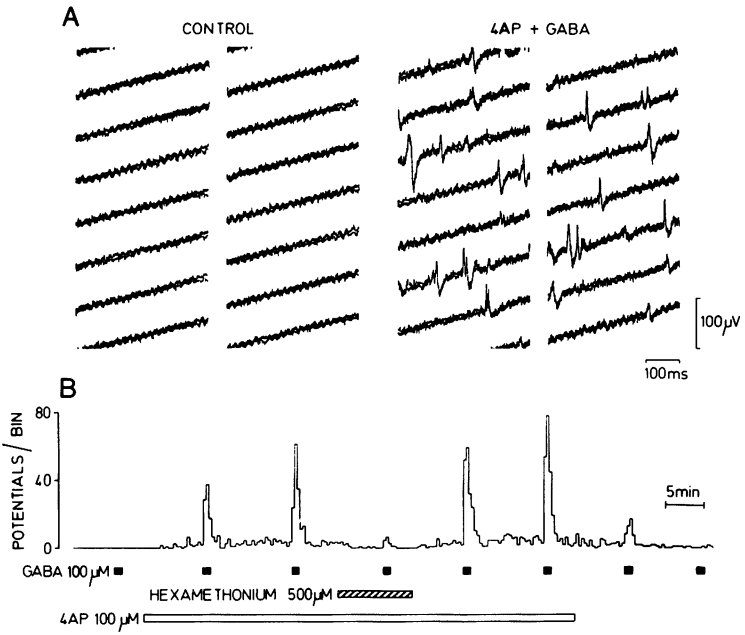

FIG. 2. Part A shows an AC-coupled recording of spontaneous potentials measured from a suction electrode containing the postganglionic sympathetic nerve. During application of 100 µmol/l GABA in 100 µmol/l 4-AP, "bursts" of postsynaptic potentials could be recorded. Part B shows the complete experiment from which the examples in A were taken. The ordinate indicates frequency of potentials (Bin width = 20 sec); abscissa time. Addition of 4-AP led to a smaller number of spontaneous potentials, the frequency of which was greatly enhanced during addition of GABA. A brief application of hexamethonium almost completely abolished this effect.

ACKNOWLEDGEMENTS

We are grateful to Ms. E. Beuschel for skilled technical assistance and to Mrs. S. Syguda for

typing the manuscript. Muscimol HCl was donated by Schering A.G., baclofen by Ciba-Geigy. This research was supported by the Deutsche Forschungsgemeinschaft, grants Br 242/14 and Br 242/16.

REFERENCES

1. Adams, P.R., and Brown, D.A. (1975): J. Physiol., 250: 85-120

2. Bowery, N.G., Doble, A., Hill, D.R., Hudson, A.L., Shaw, J.S., and Turnbull, M.J. (1979): Br. J. Pharmac., 67: 444 -445 P.

3. Brown, D.A., and Higgins, A.J. (1979): Br. J. Pharmac., 66: 108-109 P.

4. Brown, D.A., and Marsh, S. (1978): Brain Res., 156: 187-191

5. de Groat, W.C. (1970): J. Pharmacol. Exp. Ther., 172: 384-396

6. Kato, E., and Kuba, K. (1980): J. Physiol., 298: 271-283

7. Nistri, A., and Constanti, A. (1979): Progr. Neurobiol., 13: 117-235