

Picrotoxin antagonism of γ aminobutyric acid inhibitory responses and synaptic inhibition in the rat substantia nigra

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Neurons in the substantia nigra of the rat, anaesthetized with urethane, are inhibited both by electrical stimulation of the ipsilateral caudate nucleus and by iontophoretically applied γ -aminobutyric acid (GABA). Iontophoretically applied picrotoxin reversibly blocks both of these inhibitory responses. These results are consistent with the hypothesis that GABA is the transmitter released by the inhibitory striato-nigral pathway.

There is good evidence for the existence of an inhibitory pathway from the caudate nucleus to the substantia nigra (Frigyesi & Purpura, 1967; Goswell & Sedgwick, 1971; Yoshida & Precht, 1971). It seems possible that this pathway may utilize γ -aminobutyric acid (GABA) as the inhibitory transmitter. The substantia nigra contains high levels of GABA (Fahn & Côté, 1968; Okada, Nitsch-Hassler, Kim, Bak & Hassler, 1971) and glutamic acid decarboxylase, the enzyme responsible for GABA formation (Albers & Brady, 1959; Müller & Langemann, 1962). In cats, the intravenous injection of picrotoxin blocks the caudate-evoked inhibition of nigral neurones (Precht & Yoshida, 1971).

Feltz (1971) reported that neurones in the substantia nigra of cat, which were inhibited by caudate stimulation, were also inhibited by GABA. We therefore thought it of interest to investigate the effects of iontophoretically applied picrotoxin on the inhibition of substantia nigra neurones caused by caudate stimulation and iontophoretically applied GABA.

Methods.—Experiments were performed on 10 female Wistar albino rats weighing 150 g. The animals were anaesthetized with urethane (1.2–1.5 g/kg, i.p.) and placed

in a stereotaxic frame. Stimulating electrodes consisting of steel pins (insulated except for the tips) were inserted into the head of the right caudate nucleus using the co-ordinates of a stereotaxic atlas (König & Klippel, 1970) and secured to the skull with dental cement. Rectangular stimulating pulses (0.5 ms duration, 5–20 V) were supplied from a Devices gated pulse generator and stimulus isolation unit. Recordings were made from single neurones in the right substantia nigra with multibarrel glass microelectrodes to the sides of which were glued single barrel recording electrodes, arranged so that the recording barrel protruded beyond the multibarrel assembly by 10–20 μ m. The recording barrel was filled with 5 M NaCl, the other barrels containing 1.5 M NaCl, DL-homocysteic acid (0.05M, pH 8–9), GABA (1 M, pH 4) and picrotoxin (saturated in distilled water, pH 5). Using the barrel containing 1.5 M NaCl, the technique of current balancing (Salmoiraghi & Weight, 1967) was employed for the iontophoretic ejection of drugs.

Results.—All of the neurones in the substantia nigra from which recordings were made were spontaneously active and all of them were inhibited by small currents (up to 50 nA) of GABA. Picrotoxin (40 nA) was iontophoretically applied onto 20 GABA-sensitive neurones and blocked the GABA response in 19 of these cells. The effect of electrically stimulating the caudate nucleus was tested on 16 GABA-sensitive neurones. Fifteen of these cells responded to caudate stimulation with complete inhibition of firing for periods of between 100 ms and 1 second.

Picrotoxin was iontophoretically applied to 11 neurones which were inhibited both by caudate stimulation and iontophoretic GABA. On 10 of these neurones, picrotoxin was effective in blocking both the caudate-evoked and the GABA-induced inhibition. Complete blockage of the GABA response occurred within 2–3 min of picrotoxin application. Three to 10 min were required for complete blockage of the caudate-evoked inhibition.

Figure 1 shows the results obtained from one of these neurones. Traces A, C and E are rate meter recordings showing the effect on the firing rate of the cell of iontophoretically applied GABA (40 nA for 15 s). Traces B, D and F are oscilloscope traces showing the effect upon the same

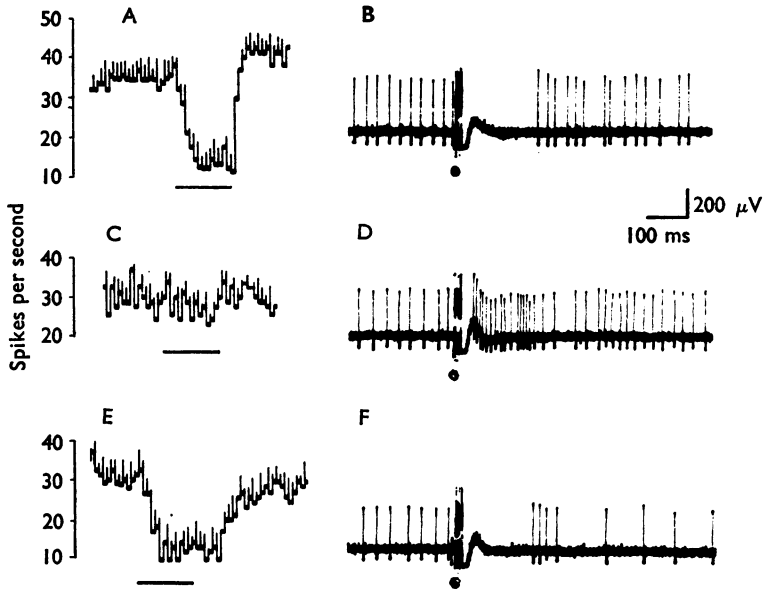


FIG. 1. Recordings taken from a single neurone in the rat substantia nigra. Traces A, C and E are rate meter recordings of cell firing frequency showing the effect of iontophoretically applied γ -aminobutyric acid (GABA) (40 nA for 15 s, indicated by solid bars). Traces B, D and F are oscilloscope traces and show the effect on spontaneous firing of stimulation of the ipsilateral caudate nucleus (3 stimuli, 0.5 ms duration at 12 V, indicated by black circles). Traces A and B, before picrotoxin; traces C and D, after 10 min application of picrotoxin; traces E and F, 10 min after picrotoxin application was discontinued.

cell of stimulating the caudate nucleus with a train of 3 stimuli (0.5 ms duration, 12 V). The top pair of traces (A and B) were recorded before application of picrotoxin and show inhibition of the cell both by iontophoretic GABA and caudate stimulation. The middle pair of traces (C and D) were taken after iontophoretic application of picrotoxin (40 nA) for 10 min and show complete abolition of the inhibition caused by both GABA application and caudate stimulation. The bottom pair of traces (E and F) were taken 10 min after the application of picrotoxin had been discontinued and show recovery of both responses.

Discussion.—The results presented in this paper confirm, in the rat, the observations made by Feltz (1971) in the cat that cells in the substantia nigra which are inhibited by electrical stimulation of the caudate nucleus are also inhibited by iontophoretically applied GABA. Furthermore, it has been shown that the inhibition of nigral neurones caused by both caudate stimulation and iontophoretically applied GABA are completely and reversibly blocked by iontophoretically applied picrotoxin. The

fact that both the drug and synaptically-evoked responses are blocked by the same antagonist is consistent with the hypothesis that GABA is the transmitter released by the inhibitory striato-nigral pathway.

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REFERENCES

- ALBERS, R. W. & BRADY, R. O. (1959). The distribution of glutamic decarboxylase in the nervous system of the rhesus monkey. *J. biol. Chem.*, **234**, 926–928.
- FAHN, S. & CÔTÉ, L. J. (1968). Regional distribution of γ -aminobutyric acid (GABA) in the brain of the rhesus monkey. *J. Neurochem.*, **15**, 209–213.
- FELTZ, P. (1971). γ -Aminobutyric acid and a caudato-nigral inhibition. *Can. J. Physiol. Pharmac.*, **49**, 1113–1115.
- FRIGYESI, T. L. & PURPURA, D. P. (1967). Electrophysiological analysis of reciprocal caudato-nigral relations. *Brain Res.* **6**, 440–456.
- GOSWELL, M. J. & SEDGWICK, E. M. (1971). Inhibition of the substantia nigra following stimulation of the caudate nucleus. *J. Physiol., Lond.*, **218**, 84–85.

- KÖNIG, J. F. R. & KLIPPEL, R. A. (1970). The rat brain. A stereotaxic atlas. Huntington, New York: Robert E. Krieger Publishing Co. Inc.
- MÜLLER, P. B. & LANGEMANN, H. (1962). Distribution of glutamic acid decarboxylase activity in human brain. *J. Neurochem.*, **9**, 399-401.
- OKADA, Y., NITSCH-HASSLER, C., KIM, J. S., BAK, I. J. & HASSLER, R. (1971). Role of γ -aminobutyric acid (GABA) in the extrapyramidal motor system. 1. Regional distribution of GABA in rabbit, rat, guinea-pig and baboon CNS. *Exp. Brain Res.*, **13**, 514-518.
- PRECHT, W. & YOSHIDA, M. (1971). Blockage of caudate-evoked inhibition of neurons in the substantia nigra by picrotoxin. *Brain Res.*, **32**, 229-233.
- SALMOIRAGHI, G. C. & WEIGHT, F. (1967). Micromethods in Neuropharmacology: An approach to the study of anaesthetics. *Anaesthesiol.*, **28**, 54-64.
- YOSHIDA, M. & PRECHT, W. (1971). Mono-synaptic inhibition of neurons of the substantia nigra by caudato-nigral fibres. *Brain Res.*, **32**, 225-228.