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Note

Simultaneous measurement of acetylcholine and dopamine releases in rat striatum under freely moving conditions with a brain dialysis method

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Acetylcholine (ACh) released from brain slices has mainly been determined by using radioisotope-labelled ACh [1,2], because the endogenously released ACh is immediately hydrolysed by acetylcholinesterase (AChE) More recently, the determination of ACh and choline (Ch) was simply and sensitively carried out by high-performance liquid chromatography (HPLC) with electrochemical detection (ED), which was selective for ACh and Ch in biological samples owing to the column separation and the use of an enzyme-immobilized column (AChE and choline oxidase) [3].

Consequently, brain dialysis methods coupled with HPLC–ED with an enzyme-immobilized column can be employed to determine released ACh in the striatum of freely moving rats [4–7] Further, in vivo brain dialysis was described after 1982 for the determination of dopamine (DA) and its metabolites [8–11] In this study, we simultaneously measured ACh and DA released in rat striatum under freely moving conditions using in vivo brain dialysis

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EXPERIMENTAL

Male Wıstar rats (250–300 g) were stereotaxıcally ımplanted wıth U-shaped mıcrodıalysıs cannulae whıle the anımals were anaesthetızed wıth pentobarbıtal (50 mg/kg) Hollow fibres (C-DAK regenerated cellulose, Japan Medıcal Supply) wıth an outer dıameter of 250 μm and a 90% cut-off of 5000 daltons were used to prepare a cannula as reported by Clemens and Phebus [8] The coordınates derıved from the atlas of Konıg and Klıppel [12] were 0 0 mm caudal to bregma, 2 7 mm lateral to mıdlıne and 6 0 mm deep to the dura (dorsal surface of skull level) After the rat had been allowed at least 1 day for surgıcal recovery, the cannula was perfused wıth Rınger solutıon contaınıng eserıne [147 mM Na^+, 2 3 mM Ca^{2+}, 4 mM K^+, 155 6 mM Cl^- and 100 μM eserıne (pH 6 0)] at a rate of 2 6 μl/mın [5,6].

ACh ın dıalysate was determıned by HPLC–ED The ACh assay method reported by Fujımorı and Yamamoto [3] was slıghtly modıfied The separatıon column was an ODS-type AC-Gel, partıcle sıze 10 μm (150×6 0 mm I D.) and the mobıle phase was 100 mM phosphate buffer (pH 8 0) contaınıng 1 mM sodıum ethylenedıamınetetraacetate (EDTA), 600 μM tetramethylammonıum chlorıde and 1 2 mM sodıum 1-decanesulphonate

The assay method for DA and ıts metabolıtes was as follows The separatıon column was also an ODS-type Inertsıl, partıcle sıze 5 μm (150×4 6 mm I D) and the mobıle phase was 100 mM cıtrate buffer (pH 4.25), contaınıng 0 1 mM EDTA, 8% acetonıtrıle and 1 2 mM sodıum 1-octanesulphonate

Electrochemıcal detectıon was carrıed out wıth a platınum workıng electrode set at 0 45 V vs Ag/AgCl and wıth a graphıte workıng electrode (ECD-100, EICOM) set at 0.65 V vs Ag/AgCl for ACh and DA assay, respectıvely The perfusate was collected at 20-mın ıntervals as one fractıon (52 μl), and a 20-μl portıon was applıed dırectly to the HPLC–ED apparatus for ACh assay For DA assay, the perfusates of the two fractıons after samplıng for ACh assay were mıxed and 50-μl portıons were applıed dırectly to the HPLC–ED system

Nomıfensıne was dıssolved ın Rınger solutıon and ınjected ınto the strıatum through the dıalysıs tube Nomıfensıne and eserıne sulphate were purchased from Hoechst Japan (Tokyo, Japan) and Sıgma (St Louıs, MO, U S A), respectıvely Other reagents were of analytıcal-reagent grade

RESULTS AND DISCUSSION

A braın dıalysıs method coupled wıth HPLC–ED usıng an enzyme-ımmobılızed column can be employed to determıne released ACh ın the strıatum of freely movıng rats Fıg. 1 shows the tıme-dependent release of ACh ın rat strıa-

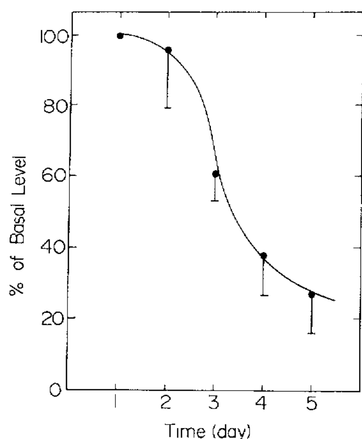

Fig 1 Time-dependent releases of ACh in rat striatum Results are means ± standard errors of the means (S E M) for four rats Basal outputs of ACh (1 day after surgery) were 5 82 ± 0 60 pmol per 20 μl per 20 min during perfusion of Ringer solution containing 10^{-4} *M* eserine

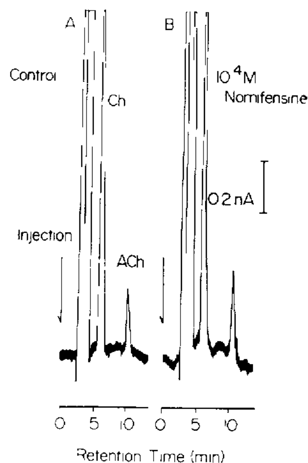

Fig 2 Chromatograms of ACh released in rat striatal dialysate with perfusion of Ringer solution containing 10^{-4} *M* eserine (A) Spontaneous output of ACh, (B) outputs of ACh with administration of 10^{-4} *M* nomifensine through the brain dialysis tube A 20-μl volume of one fraction collected for 20 min was injected into the HPLC-ED apparatus The basal levels of Ch and ACh were 8 14 and 2 88 pmol per 20 μl per 20 min, respectively

tum After the rats has been allowed 1, 2, 3, 4 and 5 days for surgical recovery, the cannula was perfused in each instance with Ringer solution containing eserine If the level of released ACh at 1 day after surgery was calculated to be 100%, the levels after 2, 3, 4 and 5 days were 95, 60, 35 and 25%, respectively This time-dependent decrease is probably the reason why various kinds of biological substances, such as proteins and lipids, are gradually adsorbed on the surface of dialysis tubes, the pores of which may then become clogged. The

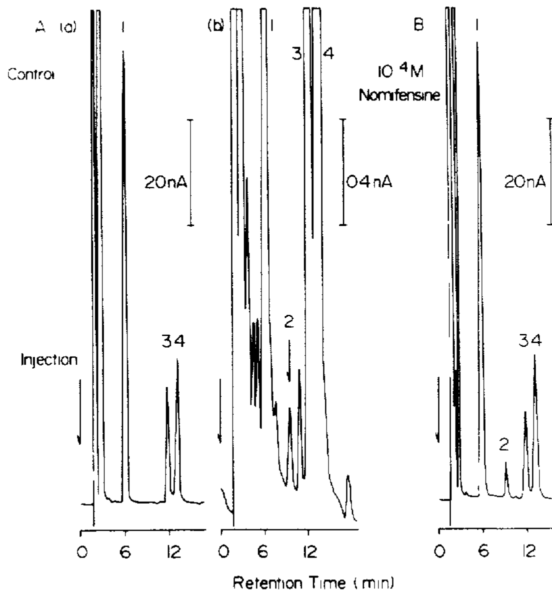

Fig 3 Chromatograms of released DA and its metabolites in rat striatal dialysate under the same conditions as in Fig 2 Peaks 1, 2, 3 and 4 represent DOPAC, DA, 5-hydroxyindoleacetic acid (5-HIAA) and HVA, respectively (A) Spontaneous outputs of DA and its metabolites, (B) outputs of DA and its metabolites with administration of nomifensine in the same manner as in Fig 2B (a), (b) A two-channel recorder was used to permit analysis of DA at different sensitivities to DOPAC, HVA and 5-HIAA A 50-μl volume of two continuous fractions was injected into the HPLC-ED apparatus

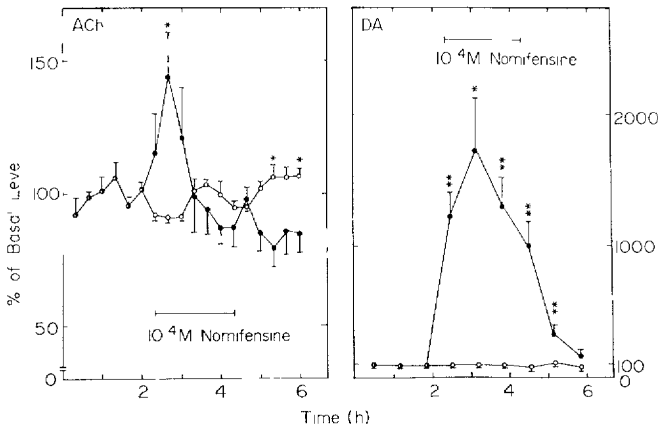

Fig 4 Effect of nomifensine on (left) ACh and (right) DA released in rat striatum Nomifensine (10^{-4} M) was administered through the dialysis tube for about 3 h Data are means $\pm$ S E M for four rats, and basal levels of ACh (six points) and DA (three points) were obtained from eight rats, respectively Basal outputs of ACh and DA were 4 36$\pm$0 76 pmol per 20 μl per 20 min, and 116$\pm$4 7 fmol per 50 μl per 40 min, respectively Open circles, control, closed circles, 10^{-4} M nomifensine

level of ACh release, however, was sufficiently stable for measurement within about 10 h, and continuous measurement within 2 days was possible Further, with replacement of the dialysis cannulae using a guide cannula, chronic measurements will be possible

Typical elution patterns of ACh and DA obtained by HPLC–ED in the dialysate of striatum are shown in Figs 2A and 3A, respectively These elution patterns were acquired from the same sample Figs 2B and 3B show the elution patterns of ACh and DA, respectively, after administration of 10^{-4} *M* nomifensine, a DA re-uptake inhibitor, through the dialysis cannula These results show that the releases of ACh and DA increased by 140 and 1800%, respectively, from the basal levels In practice, the perfusate including about 10^{-4} *M* eserine was applied directly to the HPLC–ED system to determine DA and its metabolites or ACh. The eserine retained on the DA separation column was completely washed out with 300 ml of elution buffer every day, and the eserine retained on the ACh separation column was completely washed out with 40% acetonitrile once every 2 weeks

The effects of continuous perfusion of 10^{-4} *M* nomifensine, a DA reuptake inhibitor, on the levels of released ACh and DA are shown in Fig. 4 The level of ACh released temporarily increased to 140% of the basal level, and then slightly decreased The level of DA released increased 18-fold with respect to the basal level on administration of nomifensine through the dialysis cannula The level of DA metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), released scarcely changed on administration of nomifensine

Striatal cholinergic interneurons are target cells for the nigrostriatal dopaminergic system, but the mechanism that alters striatal ACh release through activation of D_1 and/or D_2 dopamine receptors is not clear The present data indicate that ACh release is affected by dopaminergic neurons, but the mechanism may not be simple To clarify the modulation of ACh release, we studied the relationship between D_1/D_2 DA receptor and ACh release in rat striatum in vivo In spite of an 18-fold increase in DA, the levels of DA metabolites hardly increased These results may be due simply to inhibition of DA re-uptake. The side-effects of DA re-uptake inhibition (influence on DA metabolism, etc) may be the cause of these results, however Further work is required to clarify the correlation between cholinergic and dopaminergic neurons and the action of dopaminergic neurons

Recently, microdialysis has been increasingly used to determine released neurotransmitters in rat brain under freely moving conditions. These transmitters in the brain were catecholamines, ACh, amino acids and neuropeptides, and previously they were determined separately As the central nervous system is very complex, the simultaneous determination of more than two released transmitters is required In this work we simultaneously measured released ACh and DA in order to study the relationship between cholinergic and dopaminergic neurons in rat striatum. Further investigations are required of

the complex neuronal network and regulation of the neurons with this brain dialysis method under freely moving conditions

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