

the *t* complex, such as *t*<sup>12</sup>, affect the glycosylation of cell surface glycoproteins (18). Elucidation of the molecular defect in the *cld* mouse should contribute greatly to our understanding of the synthesis and relationship between these two enzymes.

The *cld* mutation may prove to be an important model for studying the clearance of plasma triglycerides since both enzymes are involved in their metabolism. The effect of the absence of either activity, as in the human syndromes, is probably modulated by the presence of the alternate pathway. In the mouse, when both enzymes are deficient, dietary fat is a lethal insult.

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## Direct in vivo Monitoring of Dopamine Released from Two Striatal Compartments in the Rat

**Abstract.** Microvoltammetric electrodes were used to monitor dopamine released in the caudate nucleus of the rat after electrical stimulation of the medial forebrain bundle. The time resolution of the technique is sufficient to determine in vivo concentration changes on a time scale of seconds. Direct evidence identifying the substance released as dopamine was obtained both voltammetrically and pharmacologically. Administration of  $\alpha$ -methyl-p-tyrosine terminates the release of dopamine, although tissue stores of dopamine are still present. Thus there appears to be a compartment for dopamine storage that is not available for immediate release. This compartment appears to be mobilized by amfonelic acid, since administration of this agent after  $\alpha$ -methyl-p-tyrosine returns the concentration of dopamine released by electrical stimulation to 75 percent of the original amount.

The understanding of the dynamics of neurotransmitter interactions in mammalian brain requires in vivo chemical sensors that are not perturbational (1). With the use of direct chemical measurements it is possible to circumvent the assumptions that are necessary with the use of labeled compounds or in vitro methods, and such direct methods should give a much more realistic measure of the important factors that regulate neurotransmission. In vivo voltammetry with microvoltammetric electrodes ( $\sim 20 \mu\text{m}$  total diameter) is one approach to this ideal since they show chemical specificity for dopamine, with a response time to concentration changes limited primarily by diffusion through brain tissue (2-4). These electrodes, when used to oxidize dopamine, give rise to voltammograms that are unique for catecholamines (3). In contrast, voltammograms for 5-hydroxytryptamine and its metabolites and metabolites of dopamine, ascorbic acid, and uric acid, all of which are also easily oxidized in mammalian brain, are poorly

defined. This difference in definition is advantageous, since direct observations of the neurotransmitter dopamine can be made without interference, as long as the concentration of this compound in extracellular fluid exceeds  $\sim 5 \mu\text{M}$ . Dayton et al. have shown that these electrodes respond in a relatively rapid fashion to changes in concentration in vivo (4). We demonstrate here that electrically stimulated dopamine release is easily monitored, that the release is dependent on the availability of dopamine and on its storage compartments, and that these factors can be altered by pharmacological agents.

In vivo electrochemical measurements were made in the caudate nucleus of male Sprague-Dawley rats anesthetized with chloral hydrate and maintained as described by Dayton et al. (4). As we and others have noted, dopamine concentrations in extracellular fluid of the rat caudate are sufficiently low that they are obscured by the other easily oxidized substances in the brain (2, 5). To in-

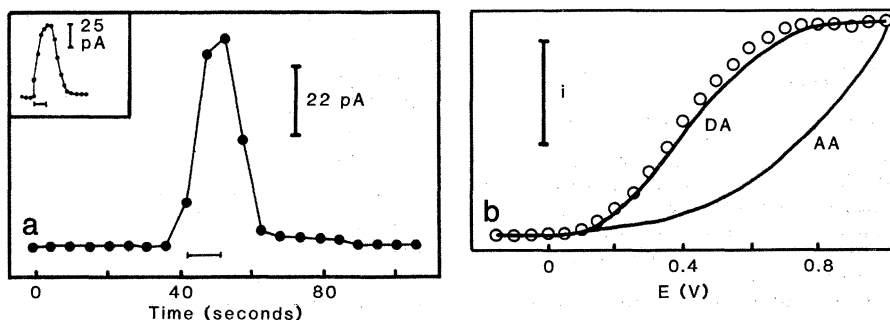


Fig. 1. Oxidation current from a microvoltammetric electrode placed in the caudate nucleus (2.4 mm anterior to bregma, 2.4 mm lateral to the midline, 4.0 mm below the dura) during a 10-second, 130- $\mu\text{A}$ , 60-Hz stimulation (horizontal bar) of the ipsilateral medial forebrain bundle. (a) Chronoamperometric current versus time at 0.5 V; 6 seconds between measurements. Inset: Chronoamperometric current versus time for an identical experiment with 2-second intervals between measured points. (b) Difference normal pulse voltammogram, the voltammogram obtained by subtracting a voltammogram before stimulation from that at the peak (circles), compared to voltammograms obtained for dopamine (DA) and ascorbic acid (AA) in vitro after the experiment (solid lines). Ascorbic acid is used for comparison because it is the predominant easily oxidized substance present in extracellular fluid of the caudate. Current (*i*) scales for the voltammograms were as follows:  $i = 16 \text{ pA}$  for  $25 \mu\text{M}$  dopamine,  $i = 28 \text{ pA}$  for  $200 \mu\text{M}$  ascorbic acid, and  $i = 32 \text{ pA}$  for stimulation. The average change in the extracellular dopamine concentration after stimulation was  $35.5 \pm 3.40 \mu\text{M}$  ( $N = 35$ ), as determined with postcalibration data. In all experiments the reference electrode was a saturated calomel electrode.

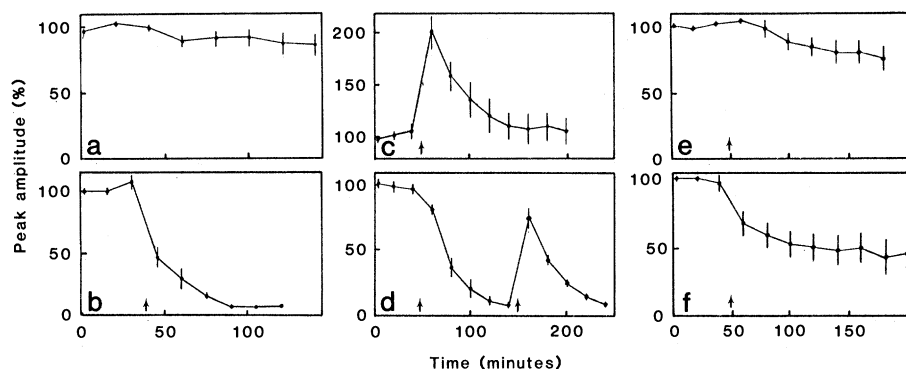


Fig. 2. Maximum current response at 0.5 V from microvoltammetric electrodes in the caudate nucleus during repetitive electrical stimulations of the medial forebrain bundle. Values are expressed as percentages of the peak amplitude compared to the values before drug administration. For each stimulation, currents were measured from the prestimulation value. All stimulations were 60 Hz, 100 to 120  $\mu$ A, and 10 seconds in duration. Drugs, administered intraperitoneally at times indicated by the arrows, were as follows: (a) control; (b)  $\alpha$ -methyl-*p*-tyrosine methyl ester (200 mg/kg); (c) amfonelic acid (5 mg/kg); (d)  $\alpha$ -methyl-*p*-tyrosine methyl ester (200 mg/kg) followed by amfonelic acid (5 mg/kg) (second arrow); (e) benztropine (25 mg/kg); and (f) *d*-amphetamine sulfate (2.5 mg/kg).

crease the extracellular concentrations of dopamine, we stimulated the medial forebrain bundle electrically with bipolar stimulating electrodes [2.2 mm posterior to bregma, 1.6 mm lateral to the midline, 8.5 mm below the dura (6); the ventral value in each rat was adjusted for maximal response]. Direct neuronal stimulation with these electrodes (60 Hz, 100 to 120  $\mu$ A root-mean-square) results in an immediate increase in the amount of oxidizable species detected by microvoltammetric electrodes implanted in the ipsilateral caudate nucleus. For stimulations of less than 10 seconds, the amount observed does not diminish during the stimulation, although a rapid disappearance of the released substance occurs after the stimulation. The voltammogram of the substance whose concentration changes is identical to that for dopamine (Fig. 1). In vivo electrochemical measurements have been made after electrical stimulation (7), but this is, as far as we know, the first voltammetric identification of electrically stimulated dopamine release. Moreover, this is the fastest time resolution of a chemical measure of catecholamine release in mammalian brain observed with any in vivo technique. Repetitive stimulations at 20-minute intervals give a constant amount of dopamine release for at least 2 hours when the duration of the stimulus is 10 seconds or less (Fig. 2a). The dopamine concentration in striatal tissue, as determined by liquid chromatography, is unchanged by this procedure.

Inhibition of dopamine synthesis with  $\alpha$ -methyl-*p*-tyrosine terminates the electrically stimulated release of dopamine (Fig. 2b). Under these conditions the striatal dopamine content is reduced (8)

but not to zero. Neuronal stimulation decreases the striatal content of dopamine after  $\alpha$ -methyl-*p*-tyrosine administration: on the nonstimulated side, dopamine concentrations are reduced to 67 percent of control ( $N = 3$ ,  $P < .03$ ) at 2 hours after the administration of  $\alpha$ -methyl-*p*-tyrosine, whereas the dopamine concentration on the stimulated side is further reduced (35 percent of control;  $N = 3$ ,  $P < .001$ ). The reduction in the total dopamine content as a result of synthesis inhibition and electrical stimulation indicates that the stimulating electrode is affecting a large number of the dopamine nerve terminals in the caudate. The absence of dopamine release, even though considerable striatal dopamine is present, is direct evidence in support of the validity of the postulate that there exist functional (releasable) and nonfunctional compartments of dopamine in the caudate (9).

Our experimental design can be used to probe further the presence of two dopamine compartments in vivo. It has been proposed that the central nervous system (CNS) actions of amfonelic acid arise because of a mobilization of the nonfunctional compartment of dopamine (10, 11). Intraperitoneal administration of amfonelic acid (5 mg/kg) increases the amount of dopamine released during electrical stimulation by 101 percent (Fig. 2c). If amfonelic acid is administered after the electrically stimulated release is inhibited by  $\alpha$ -methyl-*p*-tyrosine, the amount of dopamine released returns to 75 percent of its original value (Fig. 2d). This is the expected result for a mobilization of dopamine compartments to a releasable form, and it confirms that a nonreleasable compartment of dopa-

mine is present near the electrode after synthesis inhibition by  $\alpha$ -methyl-*p*-tyrosine.

An alternate hypothesis for the origin of the CNS actions of amfonelic acid is that it is a potent uptake inhibitor (10). To test this hypothesis the amount of dopamine released by electrical stimulation, after the introduction of recognized neuronal uptake inhibitors (12), has been compared to the effect of amfonelic acid. Intraperitoneal injection of benztropine (25 mg/kg) results in no significant change in the amount of dopamine observed after stimulation (Fig. 2e), whereas amphetamine administration (2.5 mg/kg) leads to a decrease in the amount of dopamine released (Fig. 2f). Amphetamine has many CNS actions besides blocking dopamine uptake (13). The decrease in stimulated dopamine release induced by amphetamine may occur as a result of the displacement of dopamine from storage vesicles (14) or as a result of decreased dopamine synthesis. Since the amount of stimulated dopamine release does not increase after administration of either benztropine or amphetamine but does increase dramatically after amfonelic acid administration, uptake inhibition by amfonelic acid is not likely to account for the above observations.

These in vivo data provide strong support for the idea that there are two compartments for dopamine storage in the caudate. In the "emergency" situation of electrical stimulation, the reserve compartment appears to be mobilized since striatal dopamine concentrations are decreased to a greater extent with electrical stimulation and dopamine synthesis inhibition as compared to the case for synthesis inhibition alone. However, this mobilization is not sufficient to maintain the release of dopamine except in the presence of amfonelic acid. The compartment that is sensitive to amfonelic acid appears to be a storage or reserve compartment. Amfonelic acid either facilitates transfer between compartments or facilitates direct exocytosis of dopamine stored in nonfunctional compartments. One possibility is that amfonelic acid may facilitate transport of vesicles to the synaptic membrane. Alternatively, these results may reflect the presence of two different types of dopamine nerve terminals.

The identification, by both voltammetric and pharmacological means, of dopamine as the substance released in the caudate nucleus by electrical stimulation of the medial forebrain bundle is important because it provides an in vivo method for measuring neurotransmitter dynamics. Although postmor-

tem analysis aided our interpretation of the data, the direct in vivo measurements provide new information concerning dopamine compartmentalization. The rapid clearance of dopamine confirms suggestions (2, 5) that the detection of extracellular dopamine at micromolar levels is unlikely except under extreme conditions. This work demonstrates that in vivo electrochemistry can be used to monitor the processes of neurotransmitter synthesis, storage, release, and transport, although our methods have yet to be found useful for monitoring the effects of neurotransmitter reuptake. The existence of functional and nonfunctional dopamine compartments available for exocytosis has long been hypothesized (8-11). Our data confirm the existence of these compartments and indicate that the nonfunctional compartment is available for stimulated release by the action of amfonelic acid.

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## A Polypeptide Secreted by Transformed Cells That Modulates Human Plasminogen Activator Production

**Abstract.** A diffusible factor produced and secreted by malignant murine cells was capable of inducing plasminogen activator production by normal diploid human fibroblasts. The factor's ability to induce plasminogen activator was insensitive to treatment with nucleases, but its activity was destroyed by digestion with proteases. It is proposed that such a factor would play a role in malignancy if it would recruit normal cells that were adjacent to transformed cells to produce plasminogen activator which could result in tumor-promoted proteolysis.

Plasminogen activator (PA), a serine protease, causes the conversion of plasminogen to plasmin which in turn is responsible for fibrinolysis. This process is implicated in a number of systems requiring controlled local proteolysis, including ovulation (1), embryo implantation (2), inflammation (3), cell migration and tissue remodeling (4), and the con-

version of proinsulin to insulin (5). The production and secretion of PA is also correlated with certain human disorders such as arthritis (6, 7) and the expression of transformed phenotypes (8). The enzymatic activity can be readily assayed and the use of an electrophoretic system enables distinction between species-specific forms of PA (9). We showed previously that in somatic cell hybrids between PA<sup>+</sup> mouse cells and PA<sup>-</sup> human cells, the human form of PA was expressed if human chromosome 6 was present (10). Here we show that the modulation of human PA can be mediated by a factor produced and secreted by a variety of malignant cells. The activity of this factor is insensitive to treatment with nucleases but can be destroyed with trypsin.

We initially observed modulation of human PA during attempts to map the gene for human PA. We found that production and secretion of human PA can be induced by cell fusion. Cell hybrids between PG19, a melanoma-derived PA<sup>+</sup> mouse cell line, and PA<sup>-</sup> normal diploid human fibroblasts produced mouse PA. Several of these hybrids also synthesized human PA. Since the human parental cells were PA<sup>-</sup>, we concluded that some cellular factor produced by PG19 is capable of modulating human PA.

We have examined the nature of the activation of the human PA by mouse cells. The PA was assayed by quantitative methods (3) and qualitatively by polyacrylamide gel electrophoresis (11) followed by a fibrin agar overlay technique (9). We wished to determine whether cell fusion is necessary to cause the observed PA modulation, or if the malignant murine cells were secreting a diffusible modulating factor. Actively growing human diploid fibroblasts were incubated in the presence of cell-free, serum-free conditioned culture medium from PG19. The normally PA<sup>-</sup> human fibroblasts produced high levels of PA under these conditions (Fig. 1). When the culture media or cell extracts from the two cell types were mixed and then incubated at 37°C, no human PA was



Fig. 1. Induction of PA production in normal human diploid fibroblasts. Cell sources, tissue culture, and the preparation of cell extracts were as described (10). Extracts were supplemented with sample buffer and subjected to sodium dodecyl sulfate (SDS)-polyacrylamide slab gel electrophoresis according to Laemmli (11). The gel was washed to remove SDS and was placed on an indicating agar layer containing fibrin, according to the method of Granelli-Piperno and Reich (9). After incubation at 37°C, clear bands of lysis were visible in the cloudy fibrin background, corresponding to the position of PA bands in the polyacrylamide gel. The fibrin agar layer was stained with amido black for photography. H and M mark the position of the predominant human and mouse PA bands, corresponding in size to 60,000 and 48,000 daltons, respectively. (A) PG19 cells (a line derived from a mouse melanoma). (B) Human diploid fibroblasts incubated for 17 hours with cell-free PG19 conditioned medium. (C) Human diploid fibroblasts. (D) HT1080 cells (a line derived from a human fibrosarcoma) positive for PA.