discern visually, from the final products formed, from norepinephrine (Fig. 1B), dopamine, or L-dopa. The collaborative evidence of the workers cited (1) as well as the electrophysiological data presented support the contention of Grace and Bunney that the cells in the zona compacta of the substantia nigra are good dopaminergic candidates. Thus, although the cells injected with L-dopa may well be dopaminergic, the methodologies cannot be relied upon to demonstrate this conclusively. These cells might merely be demonstrating the accumulation of L-dopa injected into their cytoplasm.

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References and Notes


2. For each substance we examined, a 10 M solution was made by dissolving the drug in a solution that consists of 25 percent saline and 25 percent glycerin in distilled water. A droplet each of the albumin solvent (control) and the albumin plus the sample was placed on either end of a glass microscope slide. The two droplets were immediately treated with a single drop (approximately 0.05 ml as measured from a 20-gauge needle and 3-ml syringe) of SPG solution (3). The slides were then dried and processed essentially as described by de la Torre and Surgeon (3). Cover slips were placed on the slides with mineral oil and the fluorescence was viewed under a Zeiss epifluorescent microscope equipped with a 50-W mercury lamp. The substances were excited through a BG-12 excitation filter and FT466 and LP478 barrier filters. The fluorescence was monitored through a Nanometrics Nanospec/105 UV-VIS-NIR microspectrophotometer attached to the camera tube of the microscope. The microspectrophotometer was controlled via linkages to a Nanometrics waveform-length programmer and a programmed Hewlett-Packard 9815A calculator to scan and plot the emission spectrum of the fluorophore automatically on a Hewlett-Packard 9817A printer.


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Keenan and Koopowitz (1) question the adequacy of methods used to establish the precise neurochemical identity of neurons from which electrophysiological recordings are made. Indeed, some investigators have recently reported recording from neurochemically identified cells when, in fact, no adequate identification had been made. However, we cannot agree with the arguments raised against our proof (2) of intracellular recordings from identified dopamine (DA)-containing neurons.

Keenan and Koopowitz argue that since L-dopa fluoresces in their experiments any cell we injected would fluoresce. However, caution must be observed when one extrapolates from data obtained in vitro in an extremely simplified solution to results obtained in vivo from chemicals injected into a complex intracellular environment in a living animal. It is not clear to us what is being measured (1). The emission maximum obtained (I) for DA, norepinephrine, and L-dopa (510 nM) is significantly higher [indeed, high enough to be confused with the characteristic serotonin yellow fluorescence of 520 nM (3)] than results obtained by others using microspectrofluorometry in brain slices or protein models [460 to 480 nM (3), the characteristic yellow-green catecholamine fluorescence we obtained with L-dopa injection]. Catecholamines and their precursors are susceptible to autooxidation at a pH above 4.0; thus the procedure used by Keenan and Koopowitz for exposing these compounds, without the protection of the cellular environment, to a pH of 7.4 at 80°C for 5 minutes in order to react them with glyoxylic acid could result in alterations of their chemical properties and may explain this discrepancy.

Keenan and Koopowitz also express concern that for some reason the amount of L-dopa injected into non-DA-containing zona reticulata (ZR) cells was inadequate in contrast to the injections into DA-containing cells. As we stated (2), the non-DA-containing ZR cells are larger and thus easier to maintain in a healthy state during intracellular recording than are the smaller and hence less stable DA neurons. Thus, the larger ejection currents and longer ejection times possible with ZR cells assured that a comparatively greater amount of L-dopa was injected into these cells than into the DA neurons. [Indeed, we have obtained consistently better results with Lucifer yellow injected into these neurons than into the DA cells (4)]. Despite these favorable experimental conditions not one fluorescent non-DA-containing ZR cell was obtained. In studies in which comparatively large amounts of L-dopa were ejected extracellularly in non-DA areas (5), no neuronal uptake was noted upon processing for fluorescence histochemistry.

The argument that the time elapsed before the rats were killed could have led to catabolism of the L-dopa in the non-DA cells seems unreasonable, since DA cells contain much higher concentrations of monoamine oxidase than non-DA cells (6). Thus the DA cells would be expected to show less fluorescence after L-dopa injection than the non-DA cells if this were the only factor operating. Since any catecholamine not taken up into granules intraneuronally is metabolized by monoamine oxidase (7), this protective granular DA uptake is another identifying characteristic of catecholamine-containing neurons. Furthermore, our fluorescing neurons cannot be epinephrine- or norepinephrine-containing cells, because the synthetic enzyme for these neurotransmitters (dopamine-beta-hydroxylase) is not present in this brain region (8). Thus, we believe our controls were adequate for this demonstration.

Nevertheless, we have used two additional histochemical procedures to increase fluorescence in intracellularly recorded DA neurons to further confirm the identity of these cells as dopaminergic. These were: (i) activation of the rate-limiting DA synthetic enzyme by intracellular injection of minute amounts of the pteridine cofactor, and (ii) intracellular injection of colchicine to increase the DA content of the DA neuron recorded. Neither the cofactor nor colchicine demonstrates fluorescence at the catecholamine emission wavelengths in vitro or in vivo. Therefore, these substances only augment fluorescence in cells already containing the biochemical machinery necessary to produce catecholamines which fluoresce with the glyoxylic acid technique (that is, in the substantia nigra, DA cells (4)).

Thus, in our view, our L-dopa results are sufficient identification of these cells as dopaminergic. This is confirmed by our other histochemical and electrophysiological methods.

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Limitations in Identifying Neurotransmitters Within Neurons by Fluorescent Histochemistry Techniques
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