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DIRECT OBSERVATION OF 6-FLUORODOPAMINE IN GUINEA PIG NERVE MICROSACS BY ^{19}F NMR

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^{19}F nuclear magnetic resonance (NMR) has been used to examine the disposition of ring-fluorinated dopamine and norepinephrine in microsacs prepared from striata of guinea pig brains. Following incubation with a $10^{-4}M$ initial concentration of 6-fluorodopamine (6F-DA), intact micmicrosacs at 4°C gave a ^{19}F NMR spectrum in which the 6F-DA present was sufficiently mobile to be visible. Intra-vesicular 6F-DA in striatal nerve terminals thus appears to exist in an environment resembling that in chromaffin vesicles but different from that prevailing inside the amine storage vesicles of platelets. Our data also suggest that the study of fluorinated compounds by ^{19}F NMR can be used to expand our understanding of processes related to amine uptake, metabolism, and storage in nerves.

The technique of nuclear magnetic resonance (NMR) has been used to explore in a non-invasive fashion the storage of neurotransmitters in vesicles of platelets, chromaffin cells, and cholinergic nerves.[1-6] These studies have been made possible by the relative purity of the preparations and by the high local concentrations of neurotransmitters prevailing inside each type of vesicle. Although large quantities of biogenic amines are also stored in central neurons, the extreme degree of heterogeneity of both the intact tissue and nerve-ending preparations makes NMR analysis difficult. We show here that these limitations can be overcome by employing ring-fluorinated analogues of amines in conjunction with ^{19}F NMR. Examination of the characteristics of 6-fluorodopamine (6F-DA) sequestered by nerve microsacs prepared from the striatum of

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guinea pig brains suggests the utility of the technique for non-invasive examination of aminergic processes both *in vitro* and *in vivo*.

MATERIALS AND METHODS

A particulate fraction of nerve-ending microsacs was prepared from the striata of nine guinea pig brains as described previously.[7,8] Microsacs in Krebs-Ringer buffer at pH 7.4 were incubated for 30 minutes at 37°C with 10^{-6}M to 10^{-3}M concentrations of fluorinated amines. Microsacs were cooled to 0°C, washed three times, and resuspended in buffer containing 20% D_2O. Perchloric acid (0.4 N) was added immediately to the washed microsacs, or intact tissue was kept at 0°C prior to NMR examination. ^{19}F NMR spectra were obtained at 338.7 mHz utilizing a Nicolet wide-bore 360 spectrometer. The pulse width was 22 μsec (= 90°), and the repetition time 1.5 sec. 6F-DA and 6-fluoronorepinephrine were synthesized as described elsewhere.[9,10]

RESULTS

2000 scans of the perchloric acid extract of microsacs prelabelled with 6F-DA were sufficient to produce an ^{19}F-NMR spectrum with a large peak corresponding in the position of its chemical shift to the 6-fluoro resonance of the parent amine (36.5 ppm with respect to hexafluorobenzene as a reference at 0 ppm) (Figure 1A). A second peak at 37.2 ppm had approximately 10% of the area of the large peak. This minor metabolite appears to be 6-fluorodihydroxyphenylacetic acid (6F-DOPAC), based on a comparison with authentic material using high-pressure liquid chromatography (HPLC).[11,12] A similar experiment, performed with 6-fluoronorepinephrine, yielded a spectrum with a large peak at 36.2 ppm for the parent amine and a smaller peak, presumably of 6-fluorodihydroxymandelic acid, at 44.0 ppm (Figure 1B).

From the ^{19}F NMR examination of the extracts, it was clearly apparent that the relatively heterogeneous microsac preparation sequestered sufficient 6F-DA to produce spectra with an adequate signal-to-noise ratio. Several factors can act to decrease the signal-to-noise ratio attainable when fluorinated amines are examined in intact biological material. These include line broadening due to field inhomogeneity, differences in the chemical environment of the amines (chemical-shift anisotropy), slow tumbling times of vesicular material, and alterations of the parent compounds during the time required for the NMR run.[1] To minimize

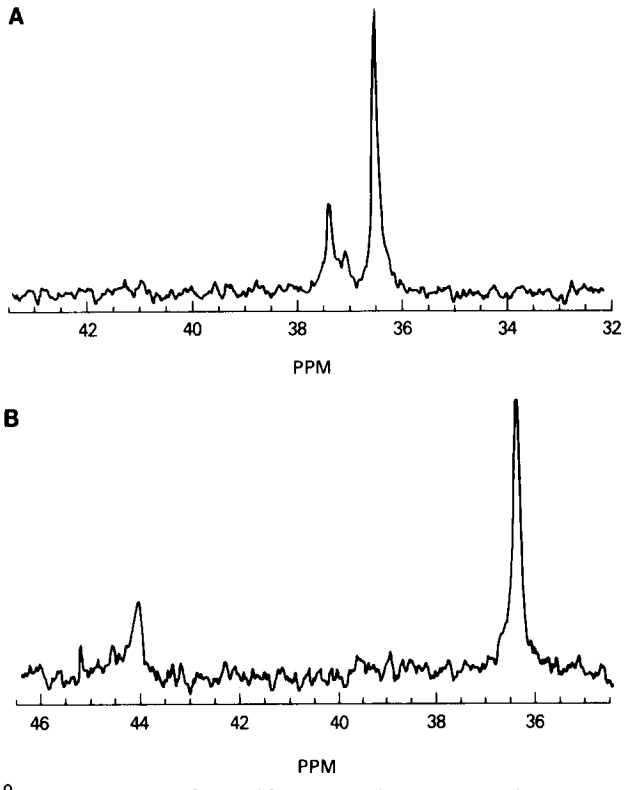

Fig. 1 ^{19}F NMR spectra of perchloric-acid extracts of nerve microsacs incubated with either 6-fluorodopamine (A) or 6-fluoronorepinephrine (B).

metabolism of 6F-DA in the intact microsac preparation, specimen temperature was kept at 4°C during the NMR run. The sample was scanned for 30,000 pulses to provide the best opportunity to observe intravesicular 6F-DA (Figure 2A). Two relatively narrow peaks at 37.1 and 44.6 ppm (62 and 65 Hz linewidths respectively) dominated the spectrum. In addition, a small 36.5 ppm peak 134 Hz in width was also observed. Raising the temperature to 10°C resulted in the disappearance of the 36.5 ppm resonance from the spectrum, even though the time elapsed during the run was only 75 minutes (3000 scans) (Figure 2B). Chromatographic analysis (HPLC) of aliquots of two samples indicated that considerable quantities of 6F-DOPAC were present along with another fluorine-containing compound, as yet not identified. The amount of 6F-DA in the extract after the 4°C run represented less than 10% of the total of both metabolites present, and was less than 5% after the 10°C run.

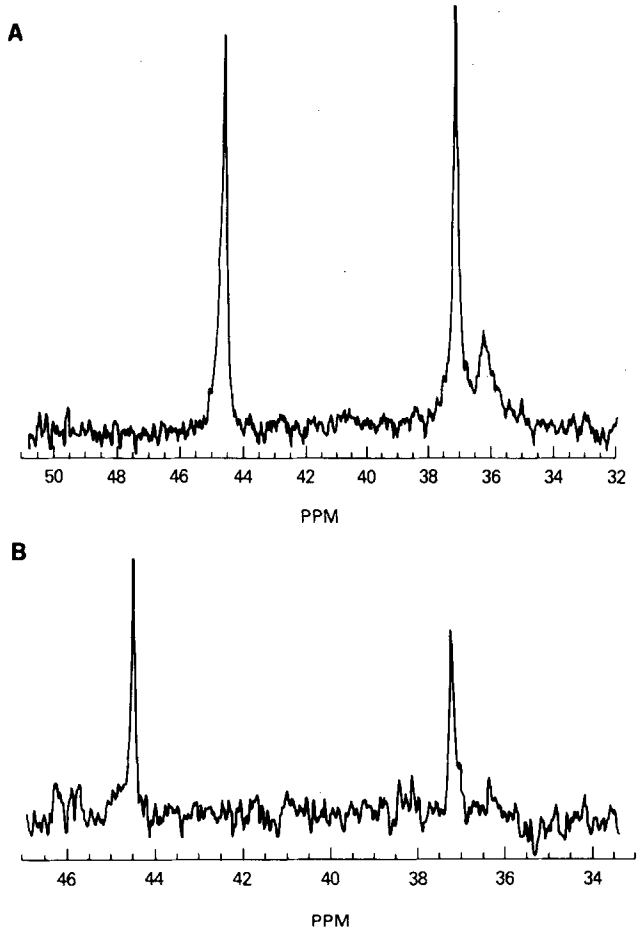

Fig. 2 ^{19}F NMR spectra of intact nerve microsacs incubated with 6-fluorodopamine and run at 4°C (A) or 10°C (B).

The NMR spectra of intact microsacs suggested that the 6F-DA present was sufficiently mobile to be observed at 4°C. However, the ratio of linewidths of 6F-DA to 6F-DOPAC (2.16) clearly indicated that 6F-DA was present in an environment in which its molecular motion was more restricted than that seen by 6F-DOPAC. We suggest that the increased linewidth observed for 6F-DA resulted from the restricted motion of 6F-DA sequestered within dense-core storage vesicles in the microsacs. The disappearance of the signal which accompanied the increase in temperature to 10°C appeared to support this suggestion, since raising the temperature would increase the metabolic rate as well as the exchange of 6F-DA with both the cytosol and the medium. Furthermore, if the microsac preparation

was pretreated with reserpine (2μM), to inhibit the uptake of 6F-DA into storage vesicles, and with pargyline (2μM), to inhibit monoamine oxidase activity prior to incubation with 6F-DA, the resulting 36.5 ppm signal had a linewidth nearly identical to that of the metabolites (ratio of 6F-DA to 6F-DOPAC, 1.18).

DISCUSSION

Our observations suggest that intra-vesicular 6F-DA in striatal nerve terminals exists in an environment with properties quite different from those seen by 5-fluorodopamine or 4,6-difluoroserotonin in the vesicles of pig platelets,[1,2] or of difluoro-serotonin in vesicles of human platelets.[1] Amine in both human and pig platelets is too restricted in motion to be visible at 4°C, although raising the temperature to 10°C gives the amine in pig platelets sufficient molecular mobility to be visible in the NMR spectrum. In both pig and human platelets, the relative immobility of intra-vesicular amine appears to be a consequence of high intra-vesicular concentrations of divalent cations and adenine nucleotides.[1,3] Since vesicles of catecholaminergic nerves have been reported to contain high levels of ATP,[13] our data suggest that these vesicles may be unique in having a relatively low content of either magnesium or calcium. In this respect they appear to resemble both chromaffin vesicles and cholinergic synaptic vesicles.[4-6]

Since the dynamics of intra-neuronal transmitter pools can be studied utilizing fluorinated compounds and ^{19}F NMR, the technique has the potential to improve our understanding of a number of aminergic processes. For example, comparisons can now be made of the relative molecular mobilities of fluorinated analogues of norepinephrine, dopamine, and serotonin in various intra-neuronal pools. Vesicular amine can be monitored during a release process induced by amphetamines or tyramines; the availability of several ring-fluorinated tyramines[9] suggests that it may be possible to follow the fate of both endogenous amine and the newly-added releasing amine. Furthermore, the technique is not limited

only to microsacs, but can readily be applied to other systems such as the pineal gland and brain-slice preparations. Continuing *in vitro* ^{19}F-NMR studies of this sort should lay the groundwork for ^{19}F NMR utilizing surface coils[14] and possibly *in vivo* monitoring of fluorinated compounds in intact animals.

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