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# PRELIMINARY COMMUNICATION

## Monoamine oxidase dependent labeling *in vivo* of mouse brain by $^{14}$C-serotonin

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IN PREVIOUS studies *in vitro* it was observed that, during incubation of labeled indoleamines with subcellular fractions from brain of rodents, radioactivity is incorporated into acid-insoluble material obtained from the incubation mixtures.[1] It was later shown that the immediate precursors for this incorporation are labeled indoleacetaldehydes derived from the corresponding amines through the action of monoamine oxidase present in the mixtures.[2] Incorporation probably involves covalent bonding, but the exact identity of polymers containing radioactive residues is not yet established. Similar phenomena were also observed *in vivo*, after i.p. administration of labeled 5-OH-tryptophan,[2] which is the biological precursor of serotonin and can cross the blood–brain barrier.[3]

In more recent studies, (3$^{1}$–$^{14}$C)-labeled serotonin was injected endocranially-intraventricularly into young adult male mice. The technique involves exposure of the skull and injection at a point 1 mm laterally and 1 mm anteriorly of the sagittal and lambdoid sutures respectively (see also ref. 4). The depth of the injection (2 mm) was regulated with the aid of a plastic sleeve placed over the needle. Under these conditions, injected material is uniformly distributed in the ventricular system as shown by injecting appropriate methylene blue solutions. The animals were sacrificed at prescribed times after injection and the brains were excised, weighed and homogenized in 10% trichloroacetic acid (20 ml, total volume). To 1-ml aliquots of the homogenates, 5 ml of 10% trichloroacetic (acid was added and the mixtures were centrifuged. The supernatants were set aside and the precipitates were washed with the acid 5 additional times by resuspension to the same volume and recentrifugation. Radioactivities in the hyamine-solubilized precipitates were determined in the scintillation counter as described previously.[5] Radioactivities in the combined supernatants (washings) were determined in the scintillation counter after mixing a 1-ml aliquot with 14·5 ml of Bray's solution.[6] Counting efficiencies were determined in all cases with the aid of internal standards.[2]

Incorporation of radioactivity following the endocranial route of administration reaches a maximum 15–30 min after injection. Later, incorporated material is gradually diminished, but radioactivity may be detected in the acid precipitates as long as 72–96 hr after injection.

Incorporation from the aldehyde level can be distinguished from stored (bound) serotonin as follows. First, incoporated material has all the physical and chemical characteristics of its counterpart obtained from studies *in vitro* (i.e. precipitation by acid, resolution in insoluble and in chloroform–methanol–soluble fractions, and subfractionation of the latter on Sephadex LH-20, as described previously[2]) and thus it differs from stored intact serotonin. Second and more important, pretreatment of the animals with various drugs influences the incorporation *in vivo* in a manner which is entirely different or opposite to what would be expected from storage. For example, in animals pretreated 24 hr earlier by a single i.p. injection of monoamine oxidase inhibitors (MAOI; e.g. pargyline, *N*-benzyl-*N*-methyl-2-propynylamine hydrochloride), incorporation is largely prevented (Table 1). Residual incorporation observed after this pretreatment is to be expected since under the above conditions *in vivo* monoamine oxidase activity cannot be completely eliminated.[7]

Most interesting is the effect of reserpine. Pretreatment with this alkaloid greatly enhances the incorporation *in vivo* (Table 1) and incorporated material remains at high levels long after the administration of labeled serotonin, practically for the duration of the manifestations of the reserpine syndrome (72–96 hr). This action of reserpine is observed only in studies *in vivo*.

Although this effect of reserpine on the incorporation could be interpreted as secondary, its primary action being interference with storage,[8, 9] the above explanation cannot account for the high levels of incorporated radioactivity as observed in reserpine-pretreated animals long after (48–96 hr) the administration of labeled serotonin (Table 1). This and other related observations will be discussed in a future publication.

The nature of soluble radioactive metabolites found in the supernatants (washings in Table 1) was studied by a combination of electrophoresis,[1] radioautography[10] and scintillation counting.[5] It was shown that 30 min after injection they consist of 5-OH-indole-3-acetic acid (70 per cent), neutral metabolites (10 per cent) and intact serotonin (20 per cent). This was true for both untreated and reserpine-pretreated animals. However, pretreatment with pargyline reversed the picture and also resulted in a decrease of neutral metabolites (i.e. from 10 to 5 per cent).

TABLE 1. INCORPORATION *IN VIVO* OF RADIOACTIVITY IN ACID-INSOLUBLE MATERIAL OBTAINED FROM MOUSE BRAIN AFTER ENDOCRANIAL-INTRAVENTRICULAR ADMINISTRATION OF LABELED SEROTONIN TO UNTREATED AND PRETREATED ANIMALS*

| Pretreatment | Radioactivity (dpm/g wet tissue) | | | |
|---|---|---|---|---|
| | Acid-insoluble | | Washings | |
| | 0·5 hr | 72 hr | 0·5 hr | 72 hr |
| 1. Untreated | 12,696 | 2304 | 232,126 | 5897 |
| 2. Pargyline | 6355 | | 376,044 | |
| 3. Reserpine | 25,332 | 10,228 | 323,906 | 9363 |
| 4. Pargyline plus reserpine | 3936 | | 306,406 | |

* Mice were pretreated by i.p. injection of either pargyline (100 mg/kg; 20 hr in advance) or reserpine (5 mg/kg; 20 hr in advance). ($3^{1}$–$^{14}$C)-labeled serotonin (0·25 μmole; 0·5 μc; 0·02 ml) was injected intraventricularly in all instances and 0·5 hr or 72 hr later the animals were sacrificed and the excised brains were processed as described in the text. In No. 4, reserpine was given 5 hr prior to the injection of the substrate to animals already pretreated (20 hr earlier) with pargyline. Processing and assays were performed on pooled acid homogenates of the brains from 5 identically treated animals in each case.

An important conclusion from such studies is that differences in total soluble radioactivities (washings in Table 1) or differences in radioactivities due to intact serotonin or 5-OH-indole-3-acetic acid (see above) are less sensitive indicators of the effect of the pretreatment (e.g. with reserpine) than differences in radioactivities of incorporated material. The latter differences then can be used as the basis of a sensitive method in studies of the action of reserpine or MAOI in relation to the metabolism of monoamines.

The occurrence of such incorporation *in vivo* in the absence of endocranially injected serotonin is surmised from observations after i.p. injection with labeled 5-OH-tryptophan.[2] Its physiological significance is not yet established. It is known[11] that in smooth muscle preparations aldehydes are completely ineffective in eliciting or inhibiting contraction. However, there are other observations indicating that aldehydes derived from neuroamines may deeply influence various metabolic activities[12, 13] and as such they may act as modulators of the function of neurons. In this respect it is of interest that many workers observed that exodenous serotonin administered endocranially to various animals produces central effects, including depression of motor activities and sedation.[14] The importance of the aldehydes may be further deduced from pronounced changes of potentials evoked by photic stimulation in rabbits after intraventricular administration of 5-OH-indole-3-acetaldehyde.*

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* In unanesthetized rabbits, monopolar recordings from the optic cortex were markedly altered in waveform, amplitude and duration 10 min after intraventricular injection of as low as 17 μg of 5-OH-indole-3-acetaldehyde, whereas the vehicle had no effect in this respect.[15]

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