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15. An LH surge is defined as two or more consecutive points exceeding the 95 percent confidence limit above baseline. Mean LH (nanograms per milliliter) released per surge was calculated as an average of the total area under the curve of each LH surge for all the animals in each group.
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Diurnal Rhythms of *N*-Acetylserotonin and Serotonin in Cerebrospinal Fluid of Monkeys

We reported a diurnal rhythm for serotonin in monkey cerebrospinal fluid (CSF) (*1*) that was suppressed by exposure to continuous light and by the β-adrenergic blocking drug, propranolol (*2*). For the serotonin assay, samples were treated with acetic anhydride prior to extraction, converting serotonin into *N*,*O*-diacetylserotonin (*1*, *3*). Any endogenous *N*-acetylserotonin (NAS) present would be converted to the same compound and would therefore subsequently be indistinguishable from serotonin.

To distinguish between the two compounds we have now modified the first stage of the derivatization process, substituting propionic anhydride for acetic anhydride as the acetylating agent (*3*). Serotonin now yields *N*,*O*-dipropionylserotonin, while NAS yields *N*-acetyl-*O*-propionylserotonin (Fig. 1).

The spirocyclic electron-capturing compounds formed by subsequent treatment with pentafluoropropionic anhydride may be distinguished on the basis of their gas-liquid chromatography (GLC) retention times as well as on the basis of the masses of their major ions. Furthermore, for serotonin only, the asymmetrical methyl group (R_2) results in the formation of two isomers that are partly separated by the capillary column, resulting in a characteristic GLC trace which further increases the certainty of identification of this compound.

Dual internal standards $^2H_4$-serotonin and $^2H_4$-NAS were used, enabling the two compounds to be quantified independently. The collection and handling of the rhesus monkey CSF samples, the remainder of the assay, and the assay of melatonin were otherwise as previously described (*1*, *2*).

Serially collected samples of CSF from seven control animals and two that had had the pineal removed have been examined. Although serotonin was detected in five of the seven controls and in one of the pinealectomized animals at concentrations of 20 to 640 pg/ml, there was no evidence of a regular nighttime rise in concentrations as previously described. Elevated serotonin concentrations were observed during the day as well as at night, and the serotonin peaks were not coincident with the daily nighttime melatonin elevations. NAS was detected in six of the seven control animals at CSF concentrations ranging from 20 to 1128 pg/ml, and in four cases a nighttime rise in levels similar to that previously ascribed to serotonin was observed. In each case in which a rhythm of NAS was detectable, melatonin also showed a rhythm, indicating the close connection between these two compounds. There was no detectable nighttime NAS in the pinealectomized animals, although both showed small amounts of NAS (up to 160 pg/ml) in daytime samples. Likewise, no nighttime melatonin elevations were observed in the CSF of the pinealectomized animals.

In light of these results, we conclude that our previous reports of nighttime elevations of serotonin in CSF were erroneous and may be principally ascribed to NAS. NAS is the limiting precursor of melatonin, with its synthesis under β-adrenergic control (*4*); it is therefore not unexpected that rhythms of NAS would be suppressed by continuous light and by propranolol (*2*). The finding of elevated nighttime levels of NAS in CSF is not in itself uninteresting, since specific brain receptors exist for this compound (*5*); it may therefore have an independent physiologic function as a pineal hormone.

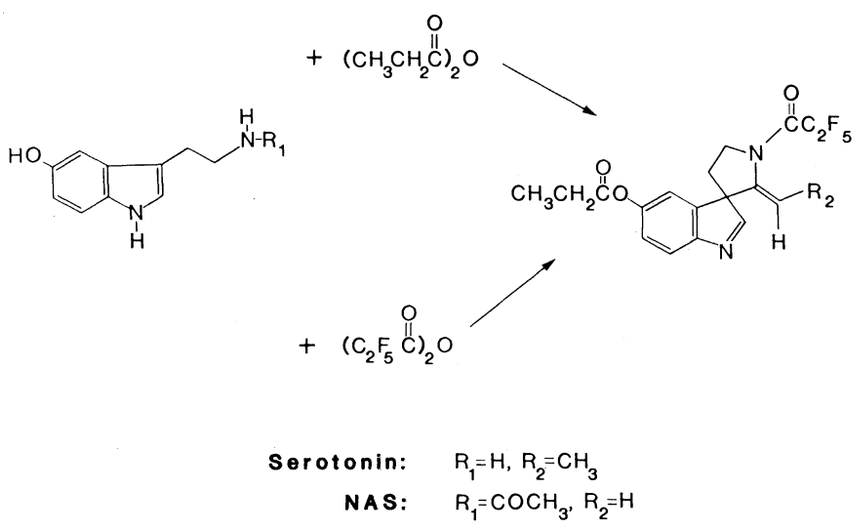

Fig. 1. Dual derivatization for serotonin and NAS.

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