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Internalization of serotonin as a consequence of its association with human platelets at 0°

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Recent work has confirmed that a small amount of labeled serotonin (5-HT) associates itself with human platelets following incubation at 4°, and it has been suggested that this phenomenon may be a manifestation of 5-HT binding to its uptake site [1]. Schick and McKean base their hypothesis on measurements of the amount of [^{3}H]-5-HT remaining with platelets after two washes, and on the fact that this amount was greatly reduced when the [^{3}H]-5-HT was added concomitantly with an excess of unlabeled 5-HT or a variety of uptake-inhibiting drugs. We have been able to replicate a portion of these findings, but we have also added observations that suggest that the "bound" 5-HT seen in washed cells may represent material actually inside the plasma membrane.

[^{3}H]-5-HT (20.2 Ci/mmole; New England Nuclear Corp., Boston, MA) at a final concentration of 10^{-7} M was added to human platelets, which were collected and washed as described previously [2]. Aliquots (500 μl) of the cells were kept at 0° and subjected to various experimental protocols (Table 1). Cells centrifuged directly after the addition of [^{3}H]-5-HT were associated with about 1 per cent of the total counts added (11.5×10^{-21} moles/platelet) (Row IA). Some of the radioactivity presumably represented material specifically bound to a 5-HT uptake site, since the addition of either 10^{-6} imipramine or a 100-fold excess of unlabeled 5-HT reduced the amount of pellet-associated [^{3}H]-5-HT to 4.2×10^{-21} moles/platelet (probably [^{3}H]-5-HT trapped passively in the pellet extracellular water) (Row IIA). As reported by Schick and McKean [1], the 7×10^{-21} moles/platelet of specifically "bound" [^{3}H]-5-HT (Row IIIA) remained with the cells after pelleting, resuspension in fresh buffer, and pelleting again (all at 0°) (Row IB). Also in accord with their observations, less than 10 per cent of this material was retained in the pellet following one cycle of washing when either imipramine or excess unlabeled 5-HT was added prior to or concomitantly with the [^{3}H]-5-HT (Row IIB). When the cells were washed once and then exposed to either 10^{-6} imipramine or 10^{-5} M unlabeled 5-HT, however, they lost only about 50 per cent of their "bound" [^{3}H]-5-HT (Row IIIB). This observation stands in marked contrast to the > 90 per cent loss seen when [^{3}H]-5-HT and uptake blockers were present together prior to washing.

Our data suggest that a significant fraction of the wash-resistant [^{3}H]-5-HT that was associated with platelets at 0° did not reside on plasma–membrane sites accessible to imipramine and unlabeled 5-HT. Since this did not appear to be the case in [^{3}H]-5-HT-containing cell suspensions prior to washing, it seems possible that, either before or during the washing procedure, surface-associated [^{3}H]-

Table 1. Evaluation of the reversibility of the binding of [^{3}H]-5-HT to intact human platelets at 0°

	Experimental protocol	Interpretation	Amount of [^{3}H]-5-HT associated with pellet after addition at an initial concentration of 10^{-7} M (moles/platelet × 10^{-21}, mean ± S.E.M.)
IA	Add [^{3}H]-5-HT⟶pellet	Specific binding + Trapping in extracellular water	11.46 ± 0.24
IIA	Add blocking agent⟶ add [^{3}H]-5-HT⟶pellet	Trapping in extracellular water	
	10^{-6} M Imipramine		4.210 ± 0.387
	10^{-5} M 5-HT		4.271 ± 0.385
IIIA	Difference IA – IIA	Specific "binding"?	7.220
IB.	Add [^{3}H]-5-HT⟶ pellet⟶ resuspend⟶ pellet	Retention of "bound" [^{3}H]-5-HT	7.041 ± 0.240
IIB.	Add blocking agent⟶ add [^{3}H]-5-HT⟶ pellet⟶ resuspend⟶ pellet	Retention of residual [^{3}H]-5-HT in extracellular H_2O?	
	10^{-6} M Imipramine		0.276 ± 0.016
	10^{-5} M 5-HT		0.529 ± 0.032
IIIB.	Add [^{3}H]-5-HT⟶ pellet⟶ resuspend⟶ add blocking agent⟶ pellet	Failure to remove "bound" [^{3}H]-5-HT	
	10^{-6} M Imipramine		5.489 ± 0.263
	10^{-5} M 5-HT		5.173 ± 0.532

5-HT moved from an accessible to an inaccessible compartment. This may occur by translocation of bound [^{3}H]-5-HT across the plasma membrane into cell cytoplasm or storage vesicles. Furthermore, it appears as if the presence of imipramine or unlabeled 5-HT during incubation at 0° and in the initial stages of the washing procedure was sufficient to prevent this transition, allowing the surface-associated [^{3}H]-5-HT to be diluted out in the fresh medium.

It is of interest that when the platelet uptake of [^{3}H]-5-HT was blocked by imipramine, resuspension of the cells in fresh medium resulted in the resumption of uptake. Under these conditions any cell-associated imipramine not covalently bound or inside the cell membrane would be expected to equilibrate with the imipramine-free buffer and be greatly reduced in concentration. Thus, the inhibitory effect of imipramine appears to be secondary to a reversible association of the molecule with the cell surface. One would expect a similar dilutional loss of any surface-associated [^{3}H]-5-HT during the two washing steps described by Schick and McKean [1], and its absence is consistent with our suggestion that the bulk of the labeled material is not accessible to the external medium. It seems possible that measurement of the imipramine-sensitive and -resistant [^{3}H]-5-HT retained after washing may provide a more useful index of 5-HT uptake sites, and of cell function, than simple measurement of the total amount of wash-resistant [^{3}H]-5-HT.

In summary, [^{3}H]-5-HT remaining associated with human platelets following incubation at 0° and one wash could not be removed when imipramine or excess 5-HT was added to the washed cells. Thus, the cell-associated [^{3}H]-5-HT does not appear to represent material bound to the external membrane surface, but rather [^{3}H]-5-HT that has been transported into the platelet.

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REFERENCES

1. P. K. Schick and M-L. McKean, *Biochem. Pharmac.* **28**, 2667 (1979).
2. J. L. Costa, D. L. Murphy and M. A. Kafka, *Biochem. Pharmac.* **26**, 517 (1977).

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Characterization of α-adrenergic receptors in rat lung membranes: presence of α_1 but not α_2 receptors

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In 1948, based on physiological studies, Ahlquist [1] suggested that adrenergic receptors be divided into α and β subtypes. About two decades later, β-adrenergic receptors were subdivided into β_1 and β_2, again on the basis of physiological experiments [2]. More recent experimental evidence has indicated the existence of subtypes of α-adrenergic receptors, i.e. α_1 and α_2 [3–5]. Using various ^{3}H-ligands, α-adrenergic receptor binding has been characterized in a variety of mammalian tissues, including brain [6, 7], kidney [6, 8], liver [9–11], platelet [12–14], uterus [15, 16], adipose tissue [17–19], heart [20] and salivary gland [21, 22]. The lung, however, has received little attention, particularly regarding the identification and characterization of its α-adrenergic receptor subtypes [23, 24].

In this communication we report the characterization of rat pulmonary α_1 adrenergic receptors. In addition, we present evidence indicating a lack of α_2-adrenergic receptors in this tissue.

Materials and methods

Randomly chosen Sprague–Dawley rats (200–300 g) were decapitated. Lungs were removed immediately, placed on ice, and then minced after discarding large bronchi. The tissue was homogenized with a Tekmar Tissumizer (model SDT) at maximum speed for two 20-sec periods (with a 10-sec interval) in 20 vol. of ice-cold 50 mM Tris–HCl (pH 8.0 at 25°). The homogenate was centrifuged at 50,000 *g* for 10 min, and the pellet was homogenized and centrifuged again. The pellet was then suspended in 30 ml of buffer and filtered through a 53-μm nylon mesh. The filtrate was centrifuged as before and the final pellet was resuspended in an appropriate volume of Tris–HCl buffer so that the final suspension contained approximately 0.2 mg protein/ml.

[^{3}H]WB4101 (24.7 Ci/mmole), [^{3}H]dihydroergocryptine (DHE) (30.4 Ci/mmole), [^{3}H]clonidine (22.2 Ci/mmole), and [^{3}H]yohimbine (81.6 Ci/mmole) were obtained from the New England Nuclear Corp. (Boston, MA). They were stored at −20° and appropriate dilutions were prepared daily just prior to use in the binding assay.

The following drugs were donated by the indicated company: (+)-norepinephrine, (+)-epinephrine, phenylephrine and (−)-isoproterenol (Sterling-Winthrop, Rensselaer, NY); phentolamine (Ciba–Geigy Corp., Summit, NJ); prazosin (Pfizer, Inc., New York, NY); clonidine (Boehringer Ingelheim, Elmsford, NY); and (−)-propranolol (Ayerst Laboratories, New York, NY). (−)-Epinephrine, (−)-norepinephrine, dopamine and yohimbine were