Meetings

Latin American Symposium on Catecholamines

Much new information on catecholamines was disclosed at the First Latin American Symposium on the subject (Buenos Aires, 3–5 August 1966). E. T. Angelakos (Boston) described a new histochemical technique for differentiating cells containing norepinephrine from cells storing epinephrine. When the catecholamine content of a tissue is liberated (by treating the tissue with tyramine, freezing it, and then thawing it, or by immersing the tissue in boiling water), the norepinephrine can be oxidized to a highly fluorescent trihydroxyindole by standard biochemical methods. Under these conditions epinephrine does not form a fluorescent product. (Both norepinephrine and epinephrine can be made to yield a fluorescent product by exposure of the tissue to formaldehyde vapor.) A different technique was described by J. H. Tramezzani (Buenos Aires). Tissues are treated with glutaraldehyde, which combines with norepinephrine to produce an insoluble compound that reduces an ammoniacal silver solution. Epinephrine apparently does not react with the glutaraldehyde. Dopamine-containing cells can be identified by exposure of tissues to higher concentrations of glutaraldehyde.

R. S. Piezzi (Mendoza) reported observations of the fine structure of chromaffin cells in the toad adrenal. This organ contains two distinct types of catecholamine cells. Cells containing norepinephrine are elongated and contain large chromaffin granules (3000 Å), pale oval nuclei, and lysosomes; they receive cholinergic innervation. Cells containing epinephrine are polygonal and contain irregular dense nuclei and smaller chromaffin granules (1500 Å); their receipt of adrenergic innervation suggests that the release of norepinephrine from the sympathetic-nerve ending controls the secretion of epinephrine from the chromaffin cell.

Also using histochemical and ultrastructural techniques, S. R. Chiocchio, A. M. Biscardi, and Tramezzani (Buenos Aires) had studied the catecholamine content of the feline carotid body. This structure also appears to contain two distinct types of chromaffin cells storing either norepinephrine or epinephrine; it also stores large amounts of dopamine (for example, half its total catecholamine content). This amine may simply serve as the precursor of the other catecholamines, or it may also be stored in a special site (perhaps within mast cells) where it may exert independent effects.

A. M. Biscardi and A. O. Donoso (Buenos Aires) had studied biosynthesis of catecholamines in the adrenal chromaffin tissue of the snake *Xenodon merremii*, in which cells containing norepinephrine or epinephrine are separated anatomically, forming two distinct zones within the gland. The norepinephrine cells are localized to a ribbon of chromaffin tissue on the periphery of the adrenal; the epinephrine-containing elements are present in the central chromaffin tissue, intermingled with cortical cells. Conversion of isotopically labeled dopa (dihydroxyphenylalanine) to epinephrine proceeded very rapidly when this precursor was incubated with chromaffin tissue from the central portion of the gland. The peripheral chromaffin tissue synthesized much smaller amounts of epinephrine; here the reaction could be enhanced by addition of adenosine triphosphate to the medium. (In similar studies Axelrod and I have observed that the methylating enzyme phenylethanolamine-*N*-methyl transferase is highly localized to the central chromaffin tissue.)

P. Delost (Clermont-Ferrand) and R. J. Wurtman and J. Axelrod (Bethesda) reported experiments on the interactions between the adrenal cortex and the adrenal medulla in the mammal. Delost had studied the effect of the medulla on the morphology and steroid content of the X-zone of the mouse adrenal cortex. (This region, which is lacking in the adult rat and human, lies directly adjacent to the adrenal medulla; it is thought to produce androgens and glucocorticoids.) It was postulated that medullary catecholamines stimulate the X-zone by a direct action.

Wurtman and Axelrod had extended previously reported studies of the control of epinephrine biosynthesis by the pituitary and the adrenal cortex; it had been shown that adrenocorticotrophic hormones (ACTH) and adrenal glucocorticoids could restore the activity of the epinephrine-forming enzyme (phenylethanolamine-*N*-methyl transferase, PNMT) in the adrenal medulla of the hypophysectomized rat. (This action probably results from increase in the amount of enzyme protein, since it can be blocked by the concurrent administration of actinomycin D or puromycin.) They had showed that the level of corticosteroid required for optimal synthesis of epinephrine was much greater than that provided by the usual "replacement dose" of hormone, but was consistant with the glucocorticoid concentration in the adrenal gland itself; thus physiologic doses of ACTH (which selectively elevate intraadrenal corticoid levels) could restore epinephrine synthesis in the hypophysectomized animal, while very large doses of glucocorticoids were needed for this purpose. When normal rats were treated with small doses of glucocorticoids (which partially suppressed the release of endogenous ACTH), PNMT activity decreased in the adrenal, suggesting that chronic treatment with small doses of corticoids may, like hypophysectomy, impair synthesis of epinephrine. Phenylethanolamine-*N*-methyl transferase was shown to exist in at least two forms, or isozymes: one, found in the rat adrenal, could be induced by steroids; the other, found in the brain, heart, and adrenal of the frog, was not altered by hypophysectomy. A. Carpi and O. A. Orsingher (Rome) presented evidence that extraadrenal chromaffin tissues may secrete small amounts of epinephrine in rats surgically deprived of their adrenal medullas.

J. Mendez and J. V. Luco (Santiago) had studied the effects of heterogenous reinnervation on the morphology and function of skeletal muscle of the cat. The longus capitis muscle was totally denervated; it normally receives cholinergic innervation. When, soon thereafter, postganglionic adrenergic fibers from the superior cervical ganglion were implanted in the muscle,

electrical stimulation of the fibers produced no electrical or mechanical response in the muscle. However, the histological changes characteristic of denervation, and the spontaneous fibrillation that usually accompanies this procedure, were markedly depressed in the vicinity of the reinnervation site.

S. Langer (Boston) discussed the relation between the potencies of certain sympathomimetic agents and the slopes of their dose-response curves, using the contraction of the cat nictitating membrane as a measure. The less-potent amines had showed steeper dose-response curves; he suggested that this effect resulted from the fact that the large doses, needed to obtain any response, saturated possible sites of uptake of catecholamine, and that this saturation then caused hypersensitivity to any norepinephrine subsequently released by the drug.

Evidence was presented by A. Pellegrino de Iraldi and L. Zieher (Buenos Aires) that dopamine exists in various tissues in an extraneuronal pool that is not lost after sympathetic denervation and which is largely unaffected by reserpine; this finding also suggests that dopamine may have many functions in the body other than serving as a precursor for norepinephrine and epinephrine. They had found the highest concentration of dopamine in any rat tissue in the pineal gland (29.9 μg/g), as well as very high levels in the caudate nucleus, the retina, the superior cervical ganglia, the adrenal gland, and the hypothalamus.

J. Daly, C. R. Creveling, and B. Witkop (Bethesda) described a rapid method of screening new compounds for ability to release norepinephrine from the heart. Mice are injected intravenously with tritiated norepinephrine; much of this is taken up by the sympathetic nerves in the heart and can be liberated by releasing agents administered 1 hour later. By use of this technique 3,5-dihydroxy-4-methoxyphenethylamine had been shown to be highly potent in releasing cardiac norepinephrine.

Direct evidence that the catecholamine content of certain brain regions is regulated by circulating steroid hormones was presented by A. O. Donoso, F. J. E. Stefano, and A. M. Biscardi (Buenos Aires). Rats were killed at various times during the vaginal estrous cycle, and their hypothalami were divided into anterior, middle, and posterior portions and assayed for norepinephrine. The level of norepinephrine in the anterior hypothalamus proved to be greatest during proestrus and fell markedly with the onset of estrus. The catecholamine content of the other hypothalamic regions was unrelated to the estrous cycle. Castration markedly increased the norepinephrine level of the anterior hypothalamus; this increase was detectable 10 days after surgery and was maximal 20 days after. Treatment with estrogen or testosterone alone did not reverse this effect of castration, but levels of norepinephrine were altered by combinations of estrogen and progesterone.

F. C. Iturriza (La Plata) described adrenergic innervation of the cells in the pars intermedia of the toad pituitary that store melanocyte-stimulating hormone (MSH). Several years ago William Etkin had demonstrated that the dispersion of pigment granules in amphibian melanophores that follows hypophysial stalk section was not terminated by regeneration of the hypophysial portal system. Restoration of the central inhibition of release of MSH required a much longer period, the length of which coincided with the time needed for regrowth of the adrenergic innervation of the pars intermedia. He suggested that the brain inhibits release of MSH not by secreting a factor into the hypophyseal portal system but by direct neural control of the pituitary cells. Iturriza's studies showed that norepinephrine-containing nerve endings terminate directly on the MSH vesicles in the pituitary cells; they provided compelling evidence that the secretion of hypothalamic "releasing factors" or "inhibiting factors" into the pituitary portal system is not the only means whereby the brain controls endocrine function. At least three endocrine organs have now been described whose parenchymal cells receive direct, adrenergic innervation: the rat pineal gland, the toad adrenal medulla, and the toad pars intermedia. It has also been suggested that monoaminergic nerve endings terminate on the beta cells of the mammalian pancreas.

G. Rodriguez de Lores Arnaiz and L. Zieher (Buenos Aires) reported studies of the subcellular distribution of adenyl cyclase, norepinephrine, dopamine, and histamine in the rat brain. Both the enzyme and the amines were found in the nerve-ending fractions. The adenyl cyclase was localized to synaptic membranes, while the amines were concentrated in a "microsomal" fraction similar to that produced by ultracentrifugation of homogenates of peripheral sympathetic nerve endings.

C. Munoz (Santiago) described techniques for relating the actions of centrally acting drugs to brain norepinephrine by studying their effects on the electroencephalogram. The area under the EEG tracing is integrated, and its modification by adrenergic drugs can be measured; synergisms and antagonisms can be demonstrated between related compounds.

Axelrod summarized present knowledge of the fate of norepinephrine and of its modifications by drugs. U. Trendelenburg (Boston) described the hypersensitivity to norepinephrine that follows sympathetic denervation or decentralization, reviewing present knowledge of the different mechanisms of these two processes. Trendelenburg and Axelrod agreed that the most important mechanism for terminating the action of "free" norepinephrine involves reuptake of the amine into sympathetic nerve endings; loss of this uptake process is mainly responsible for the hypersensitivity that follows denervation.

Marthe Vogt (Babraham) summarized current information on the role of dopamine in the basal ganglia of the brain. It was demonstrated that electrical stimulation of the substantia nigra causes the liberation of dopamine into the cerebrospinal fluid.

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Forthcoming Events

November

17–19. **Work Evaluation Units**, 3rd natl. conf., Washington, D.C. (J. E. Acker, Jr., Cardiac Work Evaluation Clinic, Knoxville, Tenn.)

17–20. American **Anthropological** Assoc., 65th annual mtg., Pittsburgh, Pa. (A. Spoehr, Dept. of Anthropology, Univ. of Pittsburgh, Pittsburgh 15213)

17–20. **Audiology**, 8th intern. congr., Mexico, D.F. (P. Berruecos Tellez, Av. Progresso 141 A, Mexico 18, D.F.)

18. Properties of **Anodized Metals and Semiconductors**, symp., Northern Electric Laboratories, Ottawa, Ont., Canada. (J. A. McDonald, Solid State Development, Northern Electric Co., R&D Labs., Box 3511, Station C, Ottawa)

18–19. **Dyslexia**, natl. conf., Philadelphia, Pa. (V. T. Keeney, 1601 Spring Garden St., Philadelphia 19130)

19. Stabilization of **Engineering and Scientific Employment in Industry**, natl. symp., San Jose State College, San Jose,