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Adrenergic neurotransmitter functions

Nobel Lecture, December 12, 1970

Chemical neurotransmission as a concept is generally attributed to Elliott[1] (1904) who emphasized the similarity between the action of adrenaline and sympathetic nerve stimulation. The experimental proof was not provided until 1921, however, by the classical experiments of Loewi[2] and by Cannon and Uridil[3]. Loewi, working on frogs, correctly concluded that the active-principle in this case was adrenaline. However, he could hardly suspect at that time that the adrenergic neurotransmitter in this species was an exception rather than the rule, and it was only some 25 years later that it became clear that the active substance which serves this function in mammals and most other animals was the nonmethylated homologue of adrenaline (Von Euler[4,5] 1946, 1948). A study of extracts of adrenergic nerves, such as the spenic nerves, and of organs supplied by such nerves, revealed certain differences between the active compound in this material and adrenaline, and with the aid of pharmacological tools and by noting certain chemical characteristics it could be identified as noradrenaline. This primary amine, which was synthesized by Stolz[6] in 1904, was independently found in extracts of the suprarenal gland by Holtz *et al.*[7] (1944/1947).

Systematic studies soon revealed that it was present in almost all organs and tissues, with the notable exception of the placenta, which is nerve-free. This suggested that its occurrence in tissues and organs depends on the presence of nerves. Section of the adrenergic nerves to the heart and some other organs and subsequent degeneration caused the noradrenaline content to fall to very low values, or to disappear, which also indicated that it was normally bound to the nerves in the organs. This concept was further supported by the finding that on regeneration of the previously sectioned nerves to the heart the noradrenaline content again rose to approximately normal values (Goodall[8]). From these observations it became apparent that the noradrenaline content of an organ or a tissue might afford an estimate of its adrenergic nerve supply. This contribution of physiology to anatomy was not entirely of a confirmatory character, since the methods available in the early fifties hardly allowed a reliable measure of the extent of sympathetic innervation to an organ or part

of an organ. By utilizing the chemical transmitter in the adrenergic nerves as a fluorogenic substance Falck and Hillarp subsequently discovered a way to visualize the individual fibres (*cf.* Falck and Torp[9]).

While it was hardly surprising that the heart or the spleen should contain considerable amounts of noradrenaline in view of their relatively rich sympathetic nerve supply, it was ofinterest to note that the lungs contained only a fraction of this amount and the skeletal muscle still much less (Table 1). On the other hand we found surprisingly large amounts of noradrenaline in the vas deferens and in the vesicular gland of the bull, suggesting either chromaffin cells or a 5-10 times richer adrenergic nerve supply than in the heart. Some years later, Sjöstrand in our laboratory in collaboration with Owman in Falck's laboratory showed the exceedingly rich adrenergic innervation to these organs (Owman and Sjöstrand[10], 1965), apparently built for sudden and vigorous contractions. This is in contrast to the testicle which almost totally lacks the sympathetic neurotransmitter.

Table 1

Noradrenaline in sheep organs

Organ	*Noradrenaline* ($\mu g/g$)
Spleen	3–4
Heart	0.6–1.1
Submaxillary gland	0.4–1.2
Kidney	0.4–0.6
Liver	0.15–0.20
Lung	0.08–0.1
Striated muscle	0.025

It soon became necessary to find methods for differentiation of adrenaline and noradrenaline in a mixture. This could be done in a simple and for most purposes satisfactory way by measuring the biological activity of the purified extracts on two test preparations with different activity quotients for the two amines, such as the cat's blood pressure and the hen's rectal caecum. From the assay results against standards the amounts of each amine could be readily calculated. These studies showed that almost every organ contained, in addition to noradrenaline, a small amount of adrenaline, which for several reasons was assumed to occur in chromaffin cells.

Using the same technique it was possible to demonstrate the large variations

in the relative noradrenaline and adrenaline contents in the suprarenals of different species, from almost no noradrenaline in the rabbit to very high proportions in the whale.

In later experiments (Folkow and Von Euler[11]) it was shown that hypothalamic stimulation caused a release of different proportions of the two amines from the adrenal glands, depending on the site of stimulus. The presence of specific noradrenaline and adrenaline cells in the adrenal medulla had previously been shown by Hillarp and Hökfelt[12].

At about the same time Goodall[8], working in our laboratory, discovered dopamine in extracts of the bovine suprarenal medulla and also in the heart, where it is located to the sinus node region (Angelakos, Fuxe and Torchianai[13]). The physiological importance of this amine as a specific agent in certain parts of the basal ganglia in the C N S has since been amply demonstrated.

After having obtained an overall picture of the distribution of the adrenergic neurotransmitter in the organism it appeared desirable to study its release, particularly since its appearance in urine, observed independently by Holtz *et al.*[7], seemed to afford a means of following this process by measuring its secretion.

During and after an intravenous infusion of adrenaline and noradrenaline, the proportion excreted in urine was small but relatively constant (Von Euler and Luft[14]). Urinary excretion of the catecholamines was therefore measured in a number of physiological and pathological conditions. It soon appeared that the noradrenaline could be used as an approximate measure of adrenergic nerve activity, whereas the adrenaline found in urine reflected the secretion from the adrenal medulla and other adrenaline-producing chromaffin cells.

The low excretion of noradrenaline during night hours and the immediate rise in the morning after standing up, as well as the maintained high level during the day suggested that the shift from horizontal to vertical position elicited increased adrenergic activity, presumably *via* the blood pressure homeostatic mechanisms. This was directly proven by subsequent studies (Sundin[15]). Muscular work was found to evoke a high activity in the adrenergic system, partly by the same mechanism (Von Euler and Hellner[16]).

It was therefore of interest to note that in postural hypotension the noradrenaline excretion was low (Luft and Von Euler[17]), whereas it was frequently increased in hypertensive states. A special condition was represented by catecholamine-producing tumours (phaeochromocytoma). The greatly increased catecholamine excretion in urine in these cases became frequently used as a method of diagnosis (Engel and Von Euler[18]).

I shall not go further into the many clinical conditions in which catecholamine excretion in urine has been measured, but I will take this opportunity to recognize with gratitude the valuable co-operation of my clinical colleagues.

Before leaving the subject of catecholamine secretion in the body I would like to touch briefly on their liberation during stress conditions. By utilizing the methods of urine catecholamine analysis, greatly furthered by the introduction of fluorimetric technique (*cf.* Ehrlén[19]), it became evident that a variety of stressful situations is accompanied by increased excretion of catecholamines, which even may serve as an indication of the degree of stress to which an individual is exposed.

In many stress situations, particularly the various kinds of emotional stress connected with pain, anxiety or apprehension, the urinary excretion pattern indicates increased adrenal medullary secretion (Bloom, Von Euler and Frankenhaeuser[20]) (Fig. 1). This field has been successfully extended by Frankenhaeuser and by Levi and their co-workers. During exposure to cold the main reaction of the organism, at least in many animals, is a greatly increased secretion of noradrenaline as shown in our laboratory by Leduc[21].

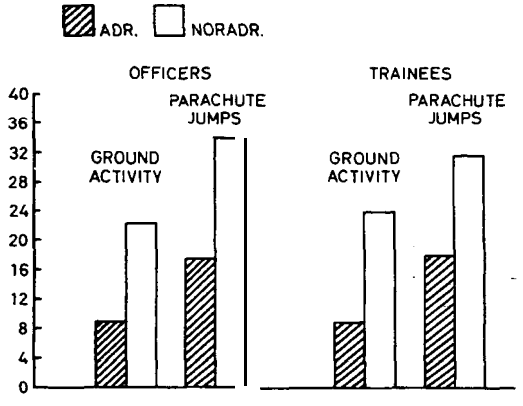

Fig. 1. Average adrenaline and noradrenaline excretion in urine in mg/min in officers and trainees during ground activity andduring a 2-3 hour period including parachute jumps. (Bloom, Von Euler and Frankenhaeuser[20])

Analysis of noradrenaline content of various organs revealed a remarkable constancy of these values. This finding raised the question as to how it was stored in the nerves. A comparison of the noradrenaline content in an organ like the spleen and in the splenic nerves indicated that the noradrenaline - assuming that it was solely confined to the nerves - must be accumulated some-

where in these. The approximate values of 10 μg noradrenaline per g nerve and 2 μg noradrenaline per g tissue would otherwise mean that 20 per cent of the spleen tissue should consist of nerves which is obviously not the case. Since the intrasplenic nerves did not deviate much from the extrasplenic nerves in their noradrenaline content it was assumed that the amine was concentrated in the nerve endings. After the pioneering work of Hillarp[22] it was known that the terminal portions of the adrenergic nerves had a beaded appearance, showing a series of swellings. We assumed therefore that these varicosities, to use the term employed by Hillarp, contained the transmitter in a high concentration. If this hypothesis was correct it appeared plausible that the transmitter should be bound to some specific structure since it was hard to believe that it should occur in a free form, in which case it was likely to diffuse out or become inactivated.

At this time two research groups (Hillarp *et al.*[23], Blaschko *et al.*[24]) had independently produced evidence to show that the adrenal medullary catecholamines were bound to subcellular particles. This might possibly be the case also for the adrenergic neurotransmitter. We therefore set out to study this question, and it could be shown that after homogenization of adrenergic nerves and various organs a small particle fraction rich in noradrenaline could be isolated (Von E uler and Hillarp[25]). In electron microscopic pictures the particles appeared as granular bodies of about 300 to 1 500 Å in diameter covered by 70 Å membrane (Fig. 2). The identification of what we believed were the specific storage structures for the adrenergic neurotransmitter seemed to provide new approaches to the problems of formation, storage and release of the transmitter. At about the same time the introduction of highly labelled catecholamines and the discovery of inactivation of the transmitter by methylation and later the reuptake phenomenon by Axelrod[26] provided new tools and concepts and induced a rapid progress in the field of adrenergic neurotransmission. This was further enhanced by important discoveries concerning the action of some drugs on the amine stores (Brodie *et al.*[27], Carlsson and Hillarp[28]).

Since sympathetic ganglia as well as the nerve trunk contained the transmitter we assumed that the storage granules were present - although in different dispersity - from the cell soma down to the terminal swellings. How did they reach the terminals? Our early suggestion (Von Euler[29]) that they might be transported by the axoplasmic flow has received strong support by the ingenious experiments by Dahlström and Häggendal[30]. The storage particles are apparently loaded with transmitter all along the axon. Synthesis is

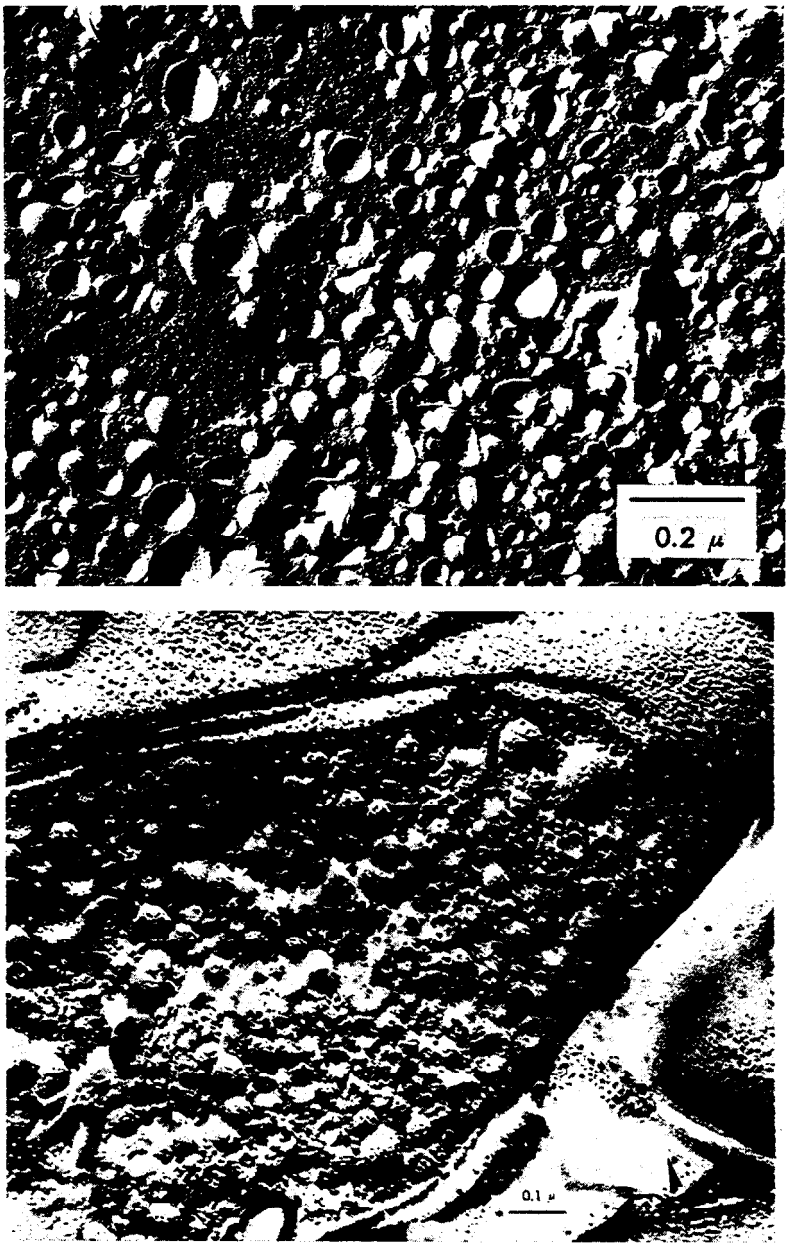

Fig. 2. Freeze-etch preparations. Upper figure: Sedimented bovine splenic nerve granules. Lower figure: Adrenergic nerve terminal swelling in guinea-pig vas deferens. (Von Euler, Gemne and Lishajko)

not confined to the presumed origin in the perikaryon, however, but proceeds at a high rate also in the axon terminals, requiring the presence of the particles for the final formation of noradrenaline from dopamine (Stjärne and Lishaj - k o[31]).

One of our early findings was that vigorous stimulation of the adrenergic nerves to the spleen did not appreciably lower the noradrenaline content of the organ in spite of considerable release (Von Euler and Hellner-Björkman[32]). From this finding we concluded that resynthesis is not only a rapid process but also that it must be regulated with great precision. We did not at that time consider the possibility of an efficient reuptake of liberated transmitter which might explain the maintenance of the stores. However, later experiments have indicated that reuptake alone could not be the cause of the undiminished stores. By administering a synthesis inhibitor, such as the boron hydride decaborane, we found that the vasoconstrictor effect of stimulating the lumbar sympathetics in the rabbit soon became greatly reduced in contrast to the effect in the untreated animal, but recovered after a period of rest (Bygdeman and Von Euler[33]) (Fig. 3). Synthesis was therefore necessary for the maintenance of the stimulation effects.

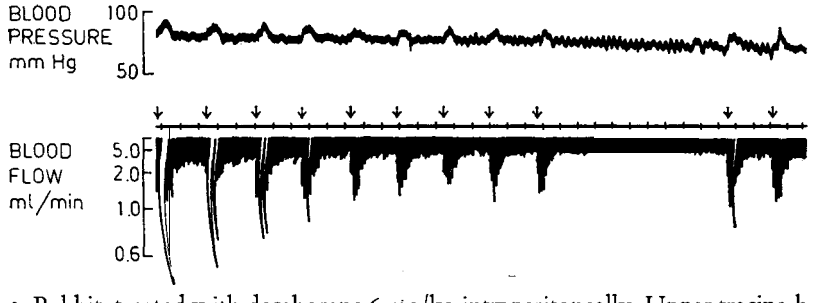

Fig. 3. Rabbit, treated with decaborane 6 mg/kg intraperitoneally. Upper tracing b pressure, lower tracing blood flow in hind leg. Declining vasoconstrictor response to electric stimulation of lumbar sympathetic trunk, 20/sec, for 30 sec, 2-min intervals. Partial recovery after prolonged interval. (Bygdeman and Von Euler[33])

Inhibition of transmitter synthesis consequently would be expected to cause depletion of the stores, as shown e.g. for decaborane. This raised the question of possible refilling of the stores by administration of exogenous noradrenaline. It proved not only possible but also occurred with remarkable ease. After an intravenous dose of noradrenaline the storage particles rapidly regained their normal content of transmitter (Von Euler and Lishajko[34]) (Fig. 4), except in the brain, to which the entrance was prevented by the blood-brain barrier.

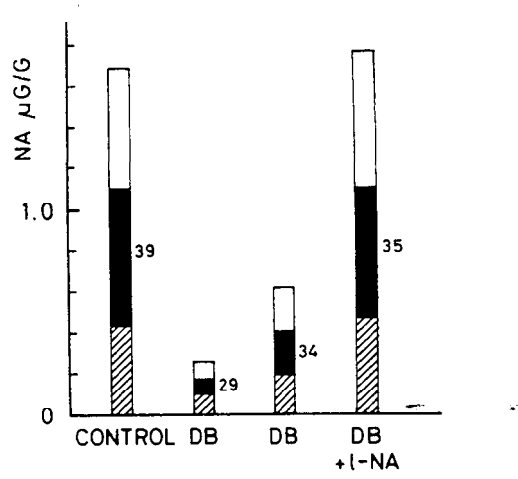

Fig. 4. Noradrenaline content in fractions of homogenized rabbit heart in controls, in decaborane-treated animals (DB) and in animals having received I-noradrenaline after depletion (+ *l* -NA). Hatched bars: Low-speed sediment (coarse particles); black bars: High-speed sediment (granules); empty bars: High-speed supernatant. (Von Euler and Lishajko[34])

The next goal was to obtain some insight in the properties of the storage particles and how they bind and release the transmitter. Since preparation of reasonably pure terminal storage particles proved very difficult we resorted to nerve trunk particles which might provide some information of value. Aided by the skill and patience of my co-worker F.Lishajko it has been possible to obtain some knowledge about their properties. As source of the particles we have used bovine splenic nerves which after homogenization and differential centrifugation yielded a preparation largely consisting of storage particles.

On incubation in phosphate buffer these were found to give off their noradrenaline at rates which depended on pH, temperature and the transmitter concentration in the medium. When bound to the particles, the noradrenaline was unaffected by oxidants like ferricyanide, indicating a complex binding by which the oxidation-sensitive groups were blocked. At low temperature release was negligible, whereas at 37°C the release was rapid with a half-time of a few minutes (Von Euler and Lishajko[35]) (Fig. 5). The high temperature dependence suggested a metabolically regulated release from the complex binding. Support for this assumption was obtained by studying the effect of various metabolic inhibitors which either blocked or enhanced the release.

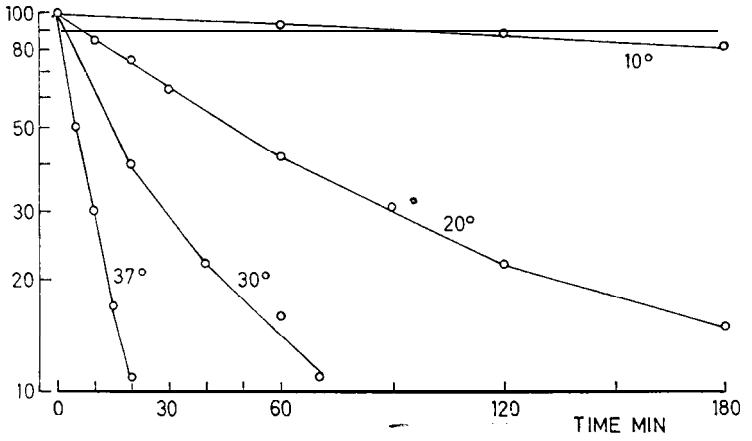

Fig. 5. Isolated bovine splenic nerve granules incubated in isotonic K-phosphate, pH 7.0 at different temperatures. Ordinate: noradrenaline content in granules in per cent of original amount. Abscissa: incubation time in minutes. (Von Euler and Lishajko[35])

With the aid of radioactively labelled noradrenaline it could be shown that uptake of transmitter occurred in the particles concomitantly with the release. This reuptake increased with increasing concentration of noradrenaline in the medium. After previous partial emptying of the amine content in the particles, a net uptake could be demonstrated during incubation with noradrenaline. Reuptake and net uptake were greatly enhanced by addition of adenosine 5′-triphosphate which again pointed at a role of this compound since it had been shown to be a natural component of the particles (Schümann[36]).

The uptake ability is not restricted to noradrenaline since adrenaline is taken up to the same extent, and α-methyl noradrenaline even more. Dopamine, on the other hand, is not specifically stored as such in the noradrenaline particles.

A large number of drugs have been found to interfere with uptake as well as release. These drugs belong to many various groups in the pharmacological arsenal, such as adrenergic blocking agents, sympathomimetic amines and psychotropic drugs, to mention a few in addition to metabolic inhibitors (Von Euler and Lishajko[37]) (Fig. 6).

The mechanisms by which the nerve impulse causes the release of the adrenergic transmitter into the receptor area of the effector cell are still incompletely understood and it remains for further work to elucidate the processes at the terminal axon membrane by which this is achieved, and at which stage the storage particles come into play, a problem studied especially by Stjärne[38]

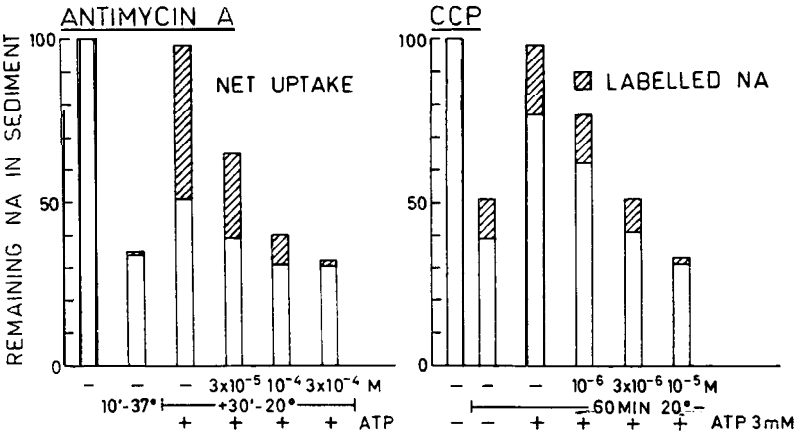

Fig. 6. Splenic nerve granules incubated in 0.13 *M* K-phosphate at pH 7.0. Left: ATP-dependent noradrenaline net uptake (column 3) in partially depleted granules, inhibited by antimycin 3 $\cdot 10^{-5}$ to 3 $\cdot 10^{-4}$ *M* (co1umn 4-6). Right: effect of exogenous ATP on noradrenaline release and reuptake (column 3) inhibited by cyano-carbonyl *m*-chloro phenylhydrazone (CCP) 10^{-6} to 3 $\cdot 10^{-6}$ *M*. Normal reuptake blocked by CCP 10^{-5} *M*. Ordinate: noradrenaline in sediment after incubation, in per cent of original amount (column 1). Abscissa: incubation time and temperature. Addition of drugs as indicated. *dl*-[^{3}H]noradrenaline 3 $\cdot 10^{-8}$ *M* added to incubation medium. Hatched parts ofcolumns, incorporated labelled noradrenaline. (Von Euler and Lishajko[37])

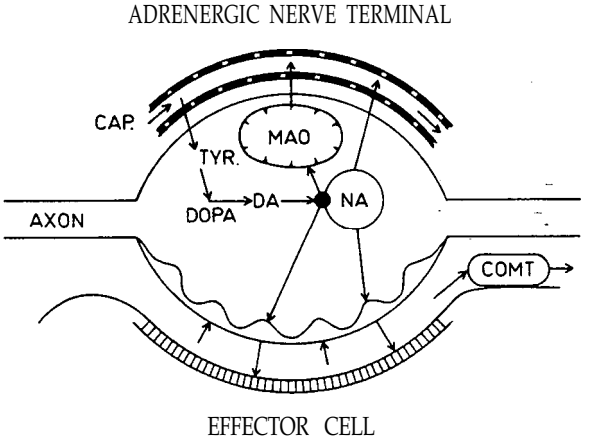

Fig. 7. Schematic drawing of adrenergic nerve terminal. Synthesis of noradrenaline (NA) requires storage granules (marked NA). Part of the newly synthesized transmitter is stored in granules, another part is transferred to a postulated membrane store from which it is released on nerve excitation. Part of the amines is oxidized by mitochondrial monoamine oxidase (MAO) or inactivated by methylation (COMT), another part is recaptured after release.

in our laboratory. Our present concept may be illustrated by the following tentative scheme (Fig. 7). The fundamental discoveries by Katz[39] on the cho - linergic neurotransmission in striated muscle may provide important clues also for the adrenergic system, as may also deeper insight in the physico-chemical shifts in the state of the membrane associated with the nerve impulse (*cf.* Nachmansohn[40]) and the possible interaction with acetylcholine (Burn and Rand[41]).

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