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SEXUAL PERFORMANCE AND LONGEVITY

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Abstract—Sexually inactive ("low-performing," LP) and highly active ("high-performing," HP) rats were selected from a sexually inexperienced population. Saline control LP rats (n = 44) lived 134.58 ± 2.29 weeks, their HP peers (n = 49) lived 151.24 ± 1.36 weeks. Life-long treatment with 0.25 mg/kg (−)deprenyl, a selective inhibitor of MAO-B that also stimulates action potential-transmitter release coupling in the catecholaminergic neurons in the brain (catecholaminergic activity enhancer, CAE, effect), enhanced the sexual and learning performance of both LP and HP rats and prolonged their life. LP rats (n = 48) treated with (−)deprenyl lived 152.54 ± 1.36 weeks and HP rats on (−)deprenyl (n = 50) lived 185.30 ± 1.96 weeks. As an indicator of the basic activity of catecholaminergic neurons, the resting release of dopamine from the striatum, substantia nigra, and tuberculum olfactorium, and of norepinephrine from the locus coeruleus, was measured in 2-, 4-, 8-, 16-, and 32-week-old male and female rats. The release of transmitters between weaning and the second month of age, i.e., during the crucial developmental phase of life, was significantly higher than either before or after that period, indicating that a CAE mechanism starts working with high intensity after weaning, lasts until the completion of full scale sexual development, and shows an unparalleled decay thereafter. It was concluded that the CAE regulation in the brain, stimulated by (−)deprenyl, controls general activity and consequently the longevity of rats.

Key Words: high-performing rat, low-performing rat, sexual performance, learning performance, longevity, (−)deprenyl, prolongation of life, brain activation, catecholaminergic activity enhancer (CAE), CAE effect

INTRODUCTION

SEXUAL PERFORMANCE of young adult male rats exhibits a very wide range of individual variation (cf. Knoll *et al.*, 1994). We selected highly sexually active/high-performing and sexually inactive/low-performing individuals from a random population of sexually inexperienced, 28-week-old males of the same breed and followed their sexual and learning performances for a lifetime. It was shown that highly sexually active rats displayed a higher learning performance and lived substantially longer than their sexually inactive peers. This study is an attempt to explain why sexually highly active males live significantly longer than their sexually inactive peers.

METHODS

Selection of sexually highly active ("high-performing," HP) and sexually inactive ("low-performing," LP) rats

Rats that did not display a single intromission during four consecutive weekly mating tests and those that showed full-scale sexual activity (mounting, intromission, and ejaculation) in each of the four tests, were selected for the study. Out of 1600 sexually inexperienced 28-week-old Wistar–Logan male rats, which were paired with a receptive female once a week during four consecutive weeks, 94 "low-performing" (LP) rats did not display a single intromission during the selection period and 99 "high-performing" (HP) males displayed at least one ejaculation in each of the four tests.

After selection the rats were treated subcutaneously, three times a week, with either 0.1 mL/100 g 0.9% NaCl or with 0.25 mg/kg (−)deprenyl, dissolved in the same volume of saline. Out of the 94 LP rats 46 were treated with saline and 48 with (−)deprenyl, in the 99 HP rats, 49 were treated with saline and 50 with (−)deprenyl. Two LP rats that died before completing their second year of life were excluded from the study.

Measurement of copulatory activity

Sexual activity of the selected males was tested once a week according to the method described previously (Knoll *et al.*, 1994).

Measurement of learning performance

The ability of the selected males to acquire a two-way conditioned avoidance response (CAR) was analyzed every three months in the shuttle box. For methodological details, see Knoll *et al.* (1994).

Measurement of orienting-searching reflex activity of rats

The orienting-searching reflex activity of hungry rats in a novel environment was measured and expressed in arbitrary units from 0 to 10 in a setup described previously (Knoll, 1969).

Measurement of norepinephrine release from the isolated brain stem of rats

The release of norepinephrine from the nerve terminals in the resting state and in response to stimulation was measured by using ^{3}H-norepinephrine according to the method described in detail previously (Knoll *et al.*, 1996c).

Measurement of the inward Ca^{2+} current in sinoatrial fibers of the frog heart

A modified version of the voltage clamping method on a double sucrose gap arrangement was used. For details, see a previous paper (Knoll *et al.*, 1996b).

RESULTS AND DISCUSSION

The difference in sexual activity between salt-treated LP and HP rats

Table 1 shows the results of the performance of LP and HP rats in copulatory behavior during a two-year test period. The males selected as sexually inactive ones remained low performers, and the males selected as highly sexually active remained high performers for a lifetime.

TABLE 1. COMPARISON OF THE MATING ACTIVITY OF VEHICLE TREATED LP AND HP RATS

		Qualification of performance (mark)				
Groups	n	*Poor (1)*	*Weak (2)*	*Medium (3)*	*Good (4)*	*Excellent (5)*
LP rats	44	43	1	—	—	—
HP rats	49	—	—	—	30	19

Rats were tested once a week in the mating test. Performance was rated according to the total number of intromission displayed by the animal during a test period of 108 weeks as follows: poor (1) <100; weak (2) 101–300; medium (3) 301–750; good (4) 751–1000; excellent >1000.

Table 2 shows in more detail the extreme differences in the sexual performance between the LP and HP groups. The average number of intromissions during the first 36 weeks of the test period was 5.43 ± 1.97 in the LP group and 420.67 ± 6.04 in the HP group. The LP rats did not display a single ejaculation during 36 weekly mating tests, while the HP rats produced 14.04 ± 0.56 ejaculations.

A comparison of the performance of the males during the first and last third of a 108-week test period shows the characteristic age-related decline of the sexual activity of male rats. The HP males, which displayed 420.67 ± 6.04 intromissions and 14.04 ± 0.56 ejaculations during the first 36-week, produced only 171.96 ± 6.90 intromissions ($p < 0.001$) and 2.47 ± 0.23 ejaculations ($p < 0.001$) during the last third of the test period.

From the data in Table 2 it is also clear that LP rats die earlier than their HP peers. Out of the 44 LP rats, only 18 were alive at the end of the two-year test period, whereas 45 of the 49 HP rats survived.

TABLE 2. COMPARISON OF THE TOTAL NUMBER OF INTROMISSIONS AND EJACULATION DISPLAYED DURING THE FIRST AND LAST THIRD OF A 108-WEEK TEST PERIOD BY VEHICLE TREATED LP AND HP RATS

	Average ± SEM of the total number of intromissions displayed				
		1–36 weeks	n	*73–108 weeks*	
LP rats	44	5.43 ± 1.97	18	1.08 ± 6.04	$p > 0.05$
HP rats	49	420.67 ± 6.04	45	171.96 ± 6.90	$p < 0.001$
	Average ± SEM of the total number of ejaculations displayed				
	n	*1–36 weeks*	n	*45*	*73–103 weeks*
LP rats	44	none	18	none	
HP rats	49	14.04 ± 0.56	45	2.47 ± 4.73	$p < 0.001$

TABLE 3. COMPARISON OF THE LEARNING ABILITY OF VEHICLE-TREATED LP AND HP RATS IN THE SHUTTLE BOX

		Qualification of performance (mark)				
Groups	n	*Poor (1)*	*Weak (2)*	*Medium (3)*	*Good (4)*	*Excellent (5)*
LP rats	44	43	1	—	—	—
HP rats	49	3	3	19	16	7

Rats were tested three monthly during of period of two years in the shuttle box, trained at 20 trials daily, for five days. The rat's performance was rated according to the total number of conditioned avoidance responses (CARs) produced by the animal during nine consecutive screenings in the shuttle box as follows: poor (1) <100; weak (2) 101–150; medium (3) 151–200; good (4) 201–250; excellent (5) >250.

Evidence That Rats Selected According to Their Copulatory Activity in Four Consecutive Mating Tests as HP or LP Rats Perform Accordingly in the Shuttle Box

Rats that proved to be sexually inactive in the weekly mating tests were also poor learners in the shuttle box, whereas the sexually high-performing males were, with very few exceptions, also high performers in the learning test. Table 3 summarizes striking difference between LP and HP rats in their ability to acquire conditioned avoidance responses (CARs). All but one of the 44 LP males were qualified as "poor"-performing rats in the shuttle box, and only 3 of 49 HP rats fitted into this category.

Table 4 presents details about the learning performance of the LP and HP rats during a two-year test period. The number of CARs in the HP group of rats decreased from 78.45 ± 3.01, displayed during the first 36 weeks, to 50.67 ± 2.99 in the last third of the test period. This is a significant age-related decay in learning performance, though less marked than the decline in sexual activity (cf. Table 2).

The 18 LP rats that survived to the end of the two-year test period had an average performance of 17.78 ± 1.79, higher than the total group had during the first 36-week period (16.36 ± 2.47), showing that the best performers survived. This is additional support for our finding that better performers live longer.

TABLE 4. COMPARISON OF THE TOTAL NUMBER OF CONDITIONED AVOIDANCE RESPONSES (CAR) DISPLAYED DURING THREE CONSECUTIVE 36-WEEK PERIODS BY VEHICLE TREATED LP AND HP RATS IN THE SHUTTLE BOX

		Average ± SEM of the total number of CARs displayed				
		1–36 weeks (1)	n	*37–72 weeks (2)*	n	*73–108 weeks (3)*
LP rats	44	16.36 ± 2.47	41	11.46 ± 1.24	18	17.78 ± 1.79
HP rats	49	78.45 ± 3.01	49	73.31 ± 2.78	45	50.67 ± 2.99
Significancy:		1:2		1:3		2:3
LP rats		$p > 0.05$		$p > 0.05$		$p < 0.01$
HP rats		$p > 0.05$		$p < 0.01$		$p < 0.001$

TABLE 5. COMPARISON OF THE MATING ACTIVITY OF (−)DEPRENYL-TREATED LP AND HP RATS

		Qualification of performance (mark)				
Groups	n	*Poor (1)*	*Weak (2)*	*Medium (3)*	*Good (4)*	*Excellent (5)*
LP rats	48	—	6	35	7	—
HP rats	50	—	—	—	—	50

For details see Table 1.

Evidence that HP rats live significantly longer than their LP peers

The salt-treated HP rats (n = 49) lived 151.24 ± 1.36 weeks, their LP peers (n = 44) lived 134.58 ± 2.29 weeks. The existence of such a highly significant ($p < 0.001$) difference in longevity between the LP and HP males suggests that a kind of a hitherto unknown "vis vitalis" mechanism in the brain controls general activity and thereby, indirectly, the lifetime of mammals. Thus, the hypothesis predicts that via this mechanism a less active, shorter living individual could be transformed into a more active, longer living one. We succeeded to demonstrate that long-term administration of (−)deprenyl, a phenylethylamine (PEA) derivative developed by us (for review see Knoll, 1995), can change a less active rat into a more active one.

(−)Deprenyl-treated HP and LP rats are sexually more active than their vehicle-treated peers

Tables 5 and 6 show that the 50 HP and 48 LP rats treated three times a week for a lifetime with 0.25 mg/kg (−)deprenyl performed significantly better in the mating test than their vehicle-treated peers (cf. Tables 1 and 2 with 5 and 6). For example: LP rats treated with (−)deprenyl displayed 239.71 ± 16.19 intromissions and 3.4 ± 0.44 ejaculations during the first 36-week test period which is, in comparison to the performance of their vehicle-treated peers (5.43 ± 1.97 intromissions and complete absence of ejaculations), a highly increased

TABLE 6. COMPARISON OF THE TOTAL NUMBER OF INTROMISSIONS AND EJACULATION DISPLAYED DURING THE FIRST AND LAST THIRD OF A 108-WEEK TEST PERIOD BY (−)DEPRENYL-TREATED LP AND HP RATS

	Average ± SEM of the total number of intromissions displayed				
	n	*1–36 weeks*	n	*73–108 weeks*	
LP rats	48	239.71 ± 16.19	44	94.61 ± 7.30	$p < 0.001$
HP rats	50	535.42 ± 5.86	50	284.28 ± 4.73	$p < 0.001$
	Average ± SEM of the total number of ejaculations displayed				
	n	*1–36 weeks*	n	*73–108 weeks*	
LP rats	48	3.40 ± 0.44	44	0.70 ± 0.15	$p < 0.001$
HP rats	50	30.04 ± 0.85	50	7.40 ± 0.32	$p < 0.001$

TABLE 7. COMPARISON OF THE LEARNING ABILITY OF (−)DEPRENYL-TREATED LP AND HP RATS IN THE SHUTTLE BOX

		Qualification of performance (mark)				
Groups	n	*Poor (1)*	*Weak (2)*	*Medium (3)*	*Good (4)*	*Excellent (5)*
LP rats	48	9	9	11	9	—
HP rats	50	—	—	—	7	43

For details see Table 3.

copulatory activity. The data show a similar difference in the sexual performance between vehicle and (−)deprenyl-treated HP rats. That (−)deprenyl treatment prolongs life of rats is evident. Of the 44 vehicle-treated LP rats only 18 survived to the end of the two-year test period, whereas of the 48 (−)deprenyl-treated LP rats 44 remained alive. Table 6 gives further proof for the characteristic, highly significant age-related decline in copulatory activity of male rats.

(−)Deprenyl-treated HP and LP rats are better performing in the shuttle box than their vehicle-treated peers

Tables 7 and 8 show that (−)deprenyl treatment substantially increased the learning ability of both HP and LP rats. The LP rats treated with (−)deprenyl were much more efficient in the shuttle box than their vehicle-treated peers. They displayed 46.85 ± 3.91 CARs during the first 36-week test period and there was no change at all in their learning performance during the two-year test period. In the HP group too, the (−)deprenyl-treated animals performed significantly ($p < 0.001$) better than the vehicle-treated HP rats (cf. Tables 4 and 8). A highly significant decline in performance with the passing of time was observed in this group of rats.

(−)Deprenyl-treated HP and LP rats live significantly longer than their vehicle-treated peers

The (−)deprenyl-treated LP rats ($n = 48$) lived 152.54 ± 1.36 weeks, i.e., significantly longer ($p < 0.001$) than their vehicle-treated peers. As a matter of fact, LP rats treated with (−)de-

TABLE 8. COMPARISON OF THE TOTAL NUMBER OF CONDITIONED AVOIDANCE RESPONSES (CARS) DISPLAYED DURING THREE CONSECUTIVE 36-WEEK PERIODS BY (−)DEPRENYL-TREATED LP AND HP RATS IN THE SHUTTLE BOX

	Average ± SEM of the total number of CARs displayed					
	n	*1–36 weeks (1)*	n	*37–72 weeks (2)*	n	*73–108 weeks (3)*
LP rats	48	46.85 ± 3.91	48	56.88 ± 2.55	44	47.00 ± 2.10
HP rats	50	113.98 ± 3.23	50	92.84 ± 2.18	50	81.68 ± 2.4

Significancy:	1:2	1:3	2:3
LP rats	$p > 0.05$	$p > 0.05$	$p < 0.01$
HP rats	$p > 0.001$	$p < 0.001$	$p < 0.001$

prenyl lived as long as the vehicle-treated HP rats (151.24 ± 1.36). The (−)deprenyl-treated HP rats ($n = 50$) lived 185.30 ± 1.96 weeks, significantly longer ($p < 0.001$) than their vehicle-treated peers.

The fact that the 48 (−)deprenyl-treated LP rats worked highly significantly better in the mating test and in the shuttle box than their vehicle-treated peers and lived as long as the 49 vehicle-treated HP rats suggests that (−)deprenyl stimulates a hitherto unknown brain mechanism that controls general activity and thereby, indirectly, the duration of life.

The action of (−)deprenyl in the mammalian brain has been described in a series of papers (Knoll, 1994, Knoll and Miklya, 1994, 1995, Knoll *et al.*, 1996a,b,c). (−)Deprenyl appears to act as a stimulant of a previously unknown catecholaminergic activity enhancer (CAE) mechanism in the brain.

The catecholaminergic activity enhancer (CAE) mechanism and its physiological significance

Of the 44 vehicle-treated LP rats that survived longer than two years, the shortest living animal died during the 116th week of its lifetime, never displayed an intromission and produced altogether three CARs during the two-year test period. In contrast, of the 49 vehicle-treated HP rats, the longest living animal died during the 170th week of its lifetime, displayed 821 intromissions, 21 ejaculations, and 311 CARs during the same period. Both were healthy males, did not differ from each other in gross behavior, and increased body weight evenly with the passing of time. To explain such physiological variations in performance and longevity between two healthy male rats of the same breed, essentials about the regulation of general activity during the developmental and postdevelopmental phase of life in the mammalian organism need to be considered.

Mammalian organisms increase their efficiency from birth to sexual maturity. Afterwards, however, due to progressive aging, performance is on a gradual decline until death. Aerobic metabolism may be responsible for age-related deterioration of living systems. Aerobic cells use their scavenger systems to protect themselves against toxic free radicals. The theory that aerobic metabolizing cells are doomed by chronic oxygen toxicity seems somewhat supported by the shortened life spans of species with higher metabolic rates.

It is known that cells of vital organs (e.g., the brain) maintain vigorous activity until death. Chronic oxygen toxicity may explain the progressive, age-related decay of organ function but cannot explain why natural death occurs when it does. An observation difficult to explain is why the organism as a whole dies while aged organs remain functional at natural death, even though the passage of time causes deterioration of parts of the system.

In mammals, the brain ensures that the organism works in a purposeful, motivated, and goal-oriented manner. Without denying the significance of the adverse consequences of natural aging in different organs, they do not compare with those age-related changes in the central nervous system (CNS). Thus, the regulation of life span may be located within the CNS.

The central hypothesis of this communication is that the catecholaminergic system is the key to understanding aging in general and CNS aging in particular. As aging progresses, the maximum activation of the CNS, via the catecholaminergic system decreases progressively. Failure of the CNS (signaled by disappearance of the electroencephalogram—EEG) occurs when the catecholaminergic system's inability to activate the higher brain centers is unable to react to stress. Hence, common problems that a younger organism can easily overcome become more problematical during aging. The essence of this hypothesis is shown in Fig. 1. According

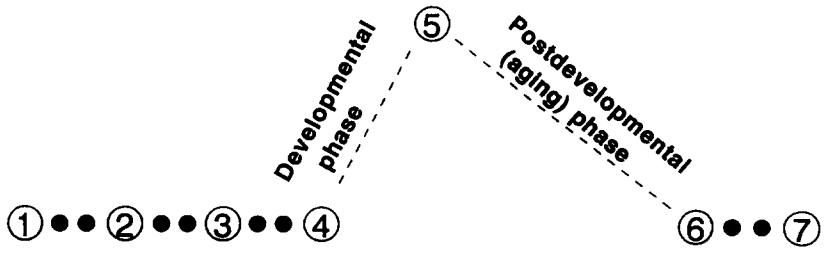

FIG. 1. Representation of the essential changes during the lifetime of mammals: 1. Fusion of the spermatozoon with the ovum. 2. The catecholaminergic system starts activating properly the higher brain levels; the integrative work of the CNS, signaled by the appearance of EEG, sets in. 3. Birth of the fetus. 4. Weaning. 5. Sexual maturity is reached. 6. Due to aging of the catecholaminergic system the integrative work of the CNS, signaled by the disappearance of the EEG, blacks out (natural death). 7. Death of the last cell.

to this hypothesis, the life of a mammalian organism can be divided into six functional stages, each one beginning with a qualitative change of crucial importance. The first stage, fertilization of the ovum, lasts until the catecholaminergic system activates the higher brain levels, which then integrates the parts of the organism into a highly sophisticated entity. This first stage of development of the mammalian organism is completed when the catecholaminergic system of the brain is activated. The appearance of the EEG signals the transition from the first into the second stage of development.

The second stage of development, birth, is the transition from fetal to postnatal circulation.

The third stage, weaning, serves to develop the skills needed for the maintenance of integrity.

The fourth stage, sexual maturity, allows for survival and maintenance of the species. Sexual maturity is the peak of developmental longevity.

Following sexual maturity the fifth, postdevelopmental (aging) stage begins.

The essence of the fifth stage is progressive decay of the efficiency of the catecholaminergic system during the postdevelopmental life span until the disappearance of the EEG occurs.

While parts of the organism remain alive, the sixth and last stage of life is the successive dying out of the different groups of cells.

The hypothesis outlined suggests that quality and duration of life rests with the inborn efficiency of the catecholaminergic brain machinery, i.e., a high-performing longer living individual has a more active, more slowly deteriorating catecholaminergic system than its low performing, shorter living peer. To simplify the concept, we may say, that a better brain engine allows better performance and a longer life span. The concept clearly predicts that, as the activity of the catecholaminergic system can be improved at any time during life, it must essentially be feasible to develop a technique for transforming a lower performing, shorter living individual, to a better performing, longer living one. It, therefore, follows that a shift of lifetime beyond the technical life span, with a yet unpredictable upper limit, may be possible in all mammals.

The hypothesis also predicts that the basic activity of the catecholaminergic system must be higher in the developmental phase of life and start to decline rapidly after sexual maturity is reached.

Hence, in our studies we have measured the resting release of dopamine from the striatum, substantia nigra, and tuberculum olfactorium, and of norepinephrine from the locus coeruleus, as an indicator of the basic activity of catecholaminergic neurons in the brain, in 2-, 4-, 8-, 16-,

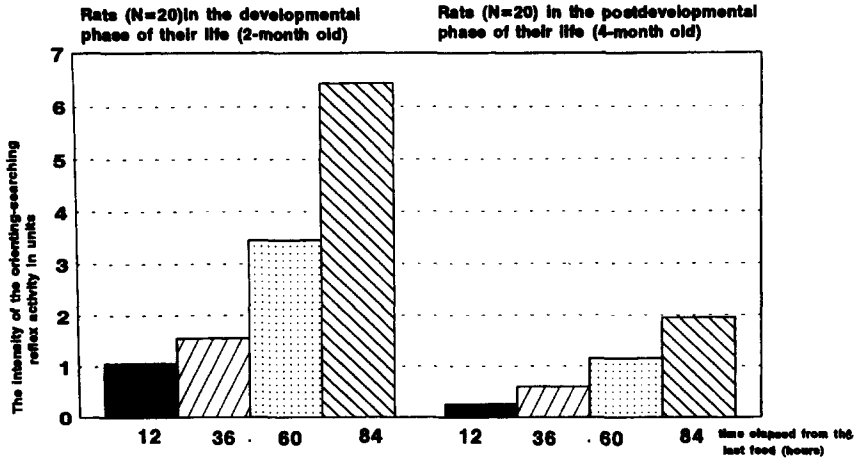

FIG. 2. The intensity of the orienting-searching reflex activity of hungry rats in a novel environment as a function of time elapsed from the last feed. Activity was measured according to Knoll (1969) and expressed in arbitrary units from 0 to 10.

and 32-week-old male and female rats. We also measured the release of serotonin from the raphe. In both male and female rats, the resting release of transmitters from brain catecholaminergic and serotoninergic neurons between weaning and the end of the second month of age, i.e., during the crucial developmental phase of their life, was significantly higher than either before or after that period, signalling a transition from a developmental to a postdevelopmental (aging) phase (Knoll and Miklya, 1995).

Hence, it is suggested that a catecholaminergic activity-enhancing mechanism starts working with high intensity after weaning, lasts until the completion of full-scale sexual development, and shows an unparalelled rate of decay thereafter. Thus, the CNS seems to be transformed, as soon as sexual maturity has been reached, by a rapid decline of catecholaminergic tone, from a "hyperactive" to an "economy" state, signalling a transition from a developmental to a postdevelopmental (aging) phase of life.

It is of critical importance that the serotoninergic system, which operates to modulate the catecholaminergic system, shows analogous changes in activity during the developmental stages of life. It appears that enhanced catecholaminergic activity terminates after sexual maturity is reached but the equilibrium of the organism is primarily regulated by this brain mechanism. Understanding how a state of enhanced catecholaminergic activity begins after weaning and terminates after sexual maturity indicates pattern of catecholaminergic activity deliberately initiated and maintained during postdevelopmental longevity. It is obvious that the catecholaminergic activity-enhancing mechanism of the brain needs to be explored in full detail.

In my interpretation, three basic modes of activity exist in the brain: a) the "active state" (assault/escape behavior, goal-seeking); b) the "vigilant resting state" (leisure); and, c) the "nonvigilant resting state" (sleeping).

The catecholaminergic system in the brain (cf. Knoll, 1969, Fig. 1) mimics the physiological needs of the vigilant resting state and the active state. Brain activity via the catecholaminergic system can be stimulated temporarily to very high activity levels. Figure 2 shows that food deprivation can induce transition from a "vigilant resting state" to an "active state." This is an

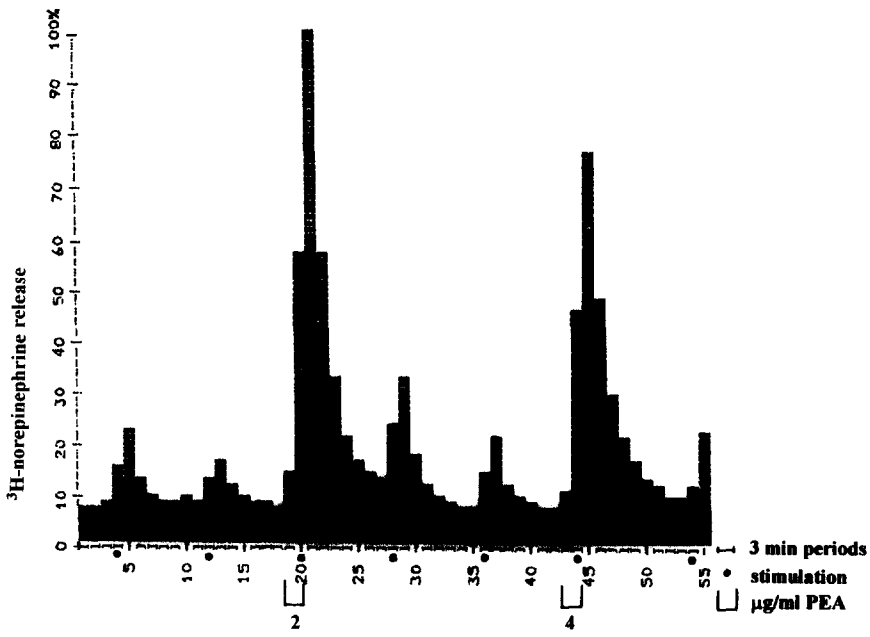

FIG. 3. Enhancement of the release of ^{3}H-norepinephrine to field stimulation (2 Hz, three minutes) from isolated rat brain stem by two consecutive doses of phenylethylamine (PEA).

example of a slowly developing and long-lasting change in the general activation of the brain. This type of brain activation evidently serves goal-seeking behavior.

The data in Fig. 2 imply an indirect proof of the striking difference in activity of the catecholaminergic system between developmental and postdevelopmental phase of life. In this experiment the intensity of the orienting-searching reflex activity of hungry rats in a novel environment was measured as a function of time elapsed since their last feeding. Rats in the late developmental phase of life (two months old) were significantly more active than those in their early postdevelopmental phase (four months old).

In contrast to goal-seeking behavior, which is the model of a slowly developing, long-lasting state of activation in the brain, assault/escape behavior needs a quite different time-scale of activation. A millisecond may only be available for transforming the resting state into maximum excitation. The catecholaminergic neurons seem to possess the ability to rapidly change their activity over a wide spectrum. As an example, Fig. 3 shows the effects of a rather low dose of phenylethylamine (PEA). PEA is highly potent in enhancing the response of the noradrenergic (NE) neuron to stimulation in the isolated brain stem and the release of ^{3}H-NE returns immediately to baseline levels upon removal of PEA from the tissue. PEA similarly enhances the release of ^{3}H-dopamine from the brain stem.

This catecholaminergic activity enhancer (CAE) effect of PEA demonstrates the ability of the catecholaminergic neurons in the brain to increase their response to stimulation (10-fold) with immediate initiation and termination. It was shown previously (Knoll *et al.*, 1996c) that the CAE effect of PEA, demonstrated in Fig. 3, is unrelated to the well-known norepinephrine displacing effect of PEA. PEA releases norepinephrine via the stoichiometric displacement of the transmitter from the neuronal stores only at high concentrations.

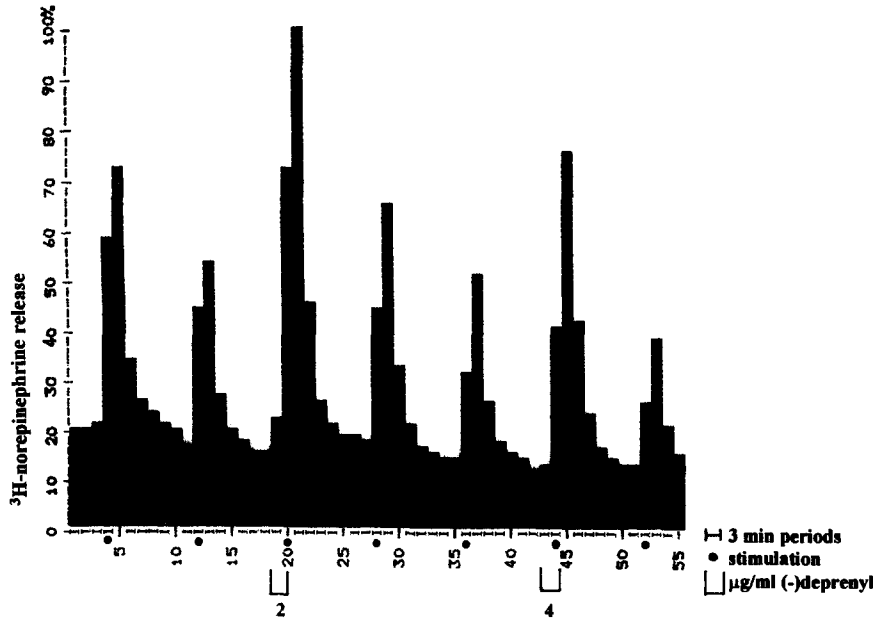

FIG. 4. Enhancement of the release of ^{3}H-norepinephrine to field stimulation (2 Hz, three minutes) from isolated rat brain stem by two consecutive doses of (−)deprenyl.

Figure 4 illustrates that (−)deprenyl (cf. Knoll, 1995) enhances the activity of the noradrenergic neurons in the brain stem in a manner similar to PEA. Both PEA and (−)deprenyl are also enhancing the release of ^{3}H-serotonin from the brain stem. This is further experimental evidence that a serotoninergic activity-enhancer mechanism, too, is operating in the brain stem, the nature of which and its relation to the CAE regulation needs clarification and is subject of experiments in progress.

The nature of the CAE effect of PEA and (−)deprenyl

The discovery that PEA and tyramine, two physiological constituents of the brain, are primarily CAE substances and displace catecholamines in much higher concentrations only (Knoll *et al.*, 1996c), and our finding that (−)deprenyl and (−)PPAP, analogues of PEA devoid of catecholamine-releasing property are sharing the CAE effect with their parent compound (Knoll *et al.*, 1996b), opened the way for a more detailed analysis and better understanding of this mechanism in the brain and in the periphery.

Theoretically (Knoll, 1994) the CAE mechanism, i.e., the rapid overexcitation of the catecholaminergic brain system in stress must mimick similar changes in the resistance arteries to respond to the enormous increased need of oxygen. We demonstrated that a kind of CAE regulation, different from that in the brain stem, works in the resistance arteries, which is stimulated by serotonin, tryptamine, PEA, and tyramine, but not by (−)deprenyl and (−)PPAP (Knoll *et al.*, 1996b).

These studies were done using the perfused central ear artery of the rabbit, in our experience, the most reliable one for studying physiological mechanisms and drug effects on resistance arteries. We were unable to detect the CAE regulation in a capacitance vessel (rabbit pulmonary

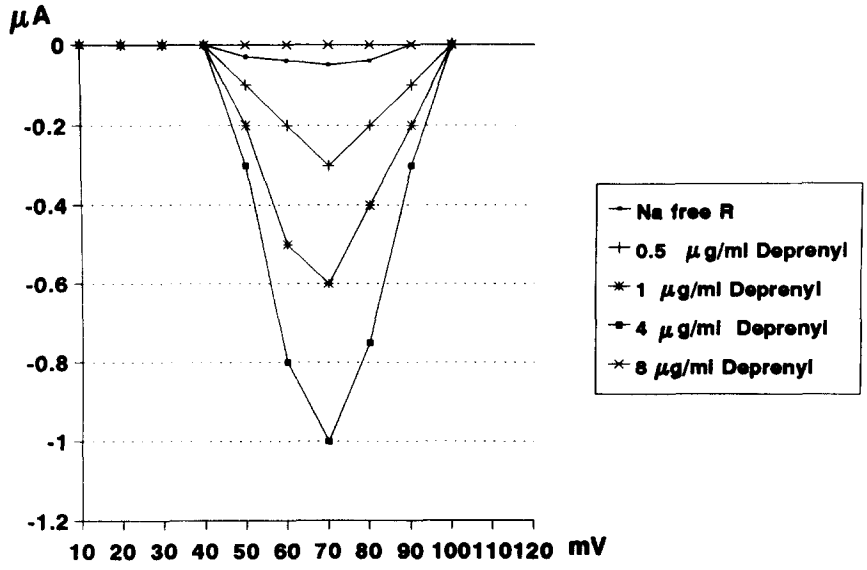

FIG. 5. Dose-related enhancement of Ca^{2+} current in sinoatrial fibers of the frog heart in Na-free Ringer (R) solution by 0.5, 1, and 4 μg/mL (−)deprenyl and the inhibitory effect of the 8 μg/mL dose (n = 3). The ±SD values are not shown in the figure because they were all less than 10%.

artery strip) and in a nonvascular norepinephrine controlled smooth muscle (isolated rat vas deferens).

The CAE mechanism operates in the sinoatrial fibers of the frog heart. In this test, small amounts of PEA, tyramine, (−)methamphetamine, (−)deprenyl, and (−)PPAP, i.e., all compounds that act as CAE substances on the isolated brain stem, at a low concentration range and dose-dependently increase the slow inward Ca^{2+} current and are inactive in higher concentration.

(−)Deprenyl acts primarily as a CAE substance in the brain (Knoll *et al.*, 1996b), and this effect is unrelated to the inhibition of MAO-B, as (−)1-phenyl-2-propylaminopentane, (−)PPAP, a deprenyl-derived substance devoid of MAO inhibitory potency, is as potent as (−)deprenyl in stimulating action potential-transmitter release coupling in the catecholaminergic neurons in the brain (Knoll *et al.* 1996b). It was also shown in this study that the CAE effect of (−)deprenyl is unrelated to inhibition of presynaptic catecholamine receptors, to the inhibition of uptake of catecholamines and to a release of catecholamines via displacement of the transmitter from the stores.

We concluded that chronic treatment with a small dose of (−)deprenyl enhances the activity of the catecholaminergic neurons in the brain (but not in the resistance arteries!) by increasing the coupling of action potential to neurotransmitter release. Thus, in the presence of (−)deprenyl a higher number of vesicles are released from the neuron in response to stimulation. Because the action potential-induced increase in cytosolic Ca^{2+} is required for the fusion of the neurotransmitter vesicles with the plasma membrane, it is reasonable to assume that (−)deprenyl acts by increasing cytosolic Ca^{2+} levels in response to stimulation. This effect of (−)deprenyl was detectable in the sinoatrial fibers of the frog heart (Fig. 5). PEA, tyramine,(−)methamphetamine and (−)PPAP acted similarly to (−)deprenyl in this test.

In the light of the new developments in the pharmacology of (−)deprenyl, it is reasonable to conclude that the CAE effect is responsible for the peculiar consequences of long-lasting treatment with this drug, that is, an aging rat performs better in the mating test and in the shuttle box and lives longer then its untreated control. Likewise it is, with all likelihood, due to the CAE effect of the drug that (−)deprenyl-treated Parkinsonian patient deteriorates slower to the stage of levodopa-need (Tetrud and Langston, 1989; Parkinson Study Group, 1989, 1993) and lives longer (Birkmayer *et al.*, 1985).

The key role of the catecholaminergic system in controlling the life-important regulatory mechanisms in the brain makes the immense indirect consequences of the CAE effect of (−)deprenyl evident. An example of a biologically not too remarkable, but statistically significant, indirect effect was presented recently by Saransaari *et al.* (1995), who found in the brain of aging rats on long-term (−)deprenyl treatment some changes in the activity of the amino acid transmitter systems. During the last years dozens of papers, the number of which is now rapidly growing, described the "neuroprotective," "trophic-like neurorescue," "apoptosis reducing," etc., effects of (−)deprenyl (e.g., Yu *et al.*, 1994; Knollema *et al.*, 1995). It remains for future research to clarify the relation of these effects to the CAE mechanism.

Regarding the mechanism of action of the CAE effect of PEA, tyramine, (−)methamphetamine, (−)deprenyl, and (−)PPAP, we assume that all these substances act via hitherto unknown, highly potent, endogenous CAE substance(s), free of catecholamine displacing property, by either mimicking its/their effect, or by activating or mobilizing such agent(s). Additional circumstantial evidence for the view that endogenous CAE substance regulate catecholaminergic activity in the brain and (−)deprenyl and (−)PPAP act via this regulation was presented in a recent paper (Knoll *et al.*, 1996a). Research is in progress to bring the question to a decision.

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