Norepinephrine Uptake into Cerebral Cortical Synaptosomes after One Fight or Electroconvulsive Shock

Abstract. *Membrane affinity for the neurotransmitter norepinephrine is rapidly but reversibly decreased in nerve terminals of the cerebral cortex by intense nervous stimulation. This should adaptively facilitate alerting during acute emergency and stress. The Michaelis constant (K_m) for the high-affinity active uptake of norepinephrine into crude synaptosome-rich homogenates of the cerebral cortices of mice was increased 68 percent after 15 minutes of intense fighting and 110 percent 5 minutes after a single electroconvulsive shock. These changes were no longer evident 18 to 20 hours later.*

Approximately 80 percent of the norepinephrine (NE) molecules released from nerve terminals in the brain are inactivated by being taken back up into neurons and sequestered in vesicular stores (*1*). The drugs that are most potent as modifiers of mood and arousal act, at least in part, by decreasing the active reuptake of NE, thereby prolonging its period of availability to postsynaptic receptors. Any alteration of reuptake occurring naturally as a consequence of neuronal activity might be expected to play a regulatory role in neurotransmission and to alter mood and the general level of arousal. Recently, we reported that increases in the apparent Michaelis constant (K_m) and maximum velocity (V_{max}) for the in vitro uptake of NE into cerebral cortical synaptosomes were evident 18 to 20 hours after the last fight in a 14-day series of short intense daily fights between male mice; this suggested that brief episodic stress had the sustained effect of causing an adaptive decrease in neuronal membrane affinity for the reuptake of NE and an adaptive increase in the number of reuptake sites (*2*). We now report that neuronal membrane affinity for NE is acutely modulated: the apparent K_m for NE uptake into synaptosomes of the cerebral cortex is very rapidly, probably immediately, increased by either a single intense episode of fighting or a single electroconvulsive shock (ECS), but these changes are no longer evident 18 to 20 hours later.

Our methods for estimating the velocity of the in vitro uptake of [^{3}H]NE and for data analysis have been described (*2*). For each analysis, the cerebral cortices of one control and one experimental mouse were homogenized gently in isotonic sucrose and crude synaptosomal fractions were prepared. Tissues were coded for "blind" analysis and processed concomitantly and identically. A series of tubes was prepared from each of the two brains, each tube containing a portion of homogenate equivalent to 5 mg of original tissue. The uptake of *dl*-[^{3}H]NE during a 5-minute incubation period at 37°C was determined concomitantly in duplicate at six substrate concentrations from 0.05 to 0.2 μM in the presence of $10^{-5} M$ pargyline, a monoamine oxidase inhibitor. The uptake at 0°C was subtracted as a blank. Mice (male, CD-1, specific pathogen free, Charles River Breeders) were made aggressive by long-term isolation and were then stimulated to fight by placing eight to ten of them together in a group (*3*). After 15 minutes of fighting, they were either killed immediately or returned to their individual cages and killed 18 to 20 hours later. Controls were isolated mice with no experience of fighting. Group-caged mice were given a single maximal tonic-clonic ECS (*4*), without anesthesia, and were either killed 5 minutes later or returned to their original cages and killed 18 to 20 hours later. Controls were treated identically, including restraint and electrode placement, except that they were not shocked.

Results observed immediately after fighting and 5 minutes after ECS are given in Table 1 and Fig. 1. The K_m and V_{max} were computed for each mouse according to Lineweaver-Burk analysis from least squares regression of the reciprocal velocity plotted against the reciprocal of substrate concentration, and treatment means for K_m and V_{max} were then derived and compared by the paired *t*-test. The V_{max} was not significantly altered in any experiment. Immediately after a single 15-minute episode of intense fighting, the apparent K_m was larger for the fighting mouse than for its control in each of seven paired comparisons. Likewise, 5 minutes after a single ECS the apparent K_m for NE uptake by cerebral cortical synaptosomes was 110 percent greater for experimental mice than for sham-shocked controls; the apparent K_m was larger for the shocked mouse than for the control in eight of nine paired

Table 1. Uptake of *dl*-[^{3}H]norepinephrine into synaptosome-rich homogenates of mouse cerebral cortex. Net uptake velocities are velocities measured after 5-minute incubations at 37°C less velocity at 0°C. Kinetic constants were derived by linear least squares regression of reciprocal velocity plotted against the reciprocal of substrate concentration. Values are the means ± standard errors of the mean. Fighting mice were grouped and allowed to fight for 15 minutes and killed immediately; they had previously been individually caged for 3 months to make them aggressive (N = seven pairs). Electroconvulsive shock was maximal and mice were killed 5 minutes later; they had previously been group-caged (N = nine pairs). By Wilcoxon sign-ranked test, the significance of the difference in K_m produced by fighting is $P < .02$, and by ECS is $P < .01$.

Mice	Net velocity (nmole per gram per 5 minutes) of uptake of *dl*-[^{3}H]NE at							Derived kinetic constants	
	0.05 μM	0.06 μM	0.075 μM	0.1 μM	0.15 μM	0.2 μM		Apparent K_m (μM)	V_{max} (nmole per gram per 5 minutes)
				Fighting					
Control	0.24 ± 0.016	0.28 ± 0.018	0.32 ± 0.021	0.38 ± 0.025	0.52 ± 0.035	0.64 ± 0.043		0.236 ± 0.025	1.47 ± 0.24
Fighting	0.20 ± 0.007	0.24 ± 0.014	0.29 ± 0.015	0.35 ± 0.015	0.49 ± 0.021	0.62 ± 0.029		0.396 ± 0.068	1.88 ± 0.24
							t*	2.58	1.33
							P	< .05	> .05
				Electroconvulsive shock					
Control	0.23 ± 0.017	0.27 ± 0.020	0.31 ± 0.021	0.39 ± 0.025	0.53 ± 0.037	0.66 ± 0.047		0.339 ± 0.035	1.87 ± 0.22
ECS	0.19 ± 0.006	0.23 ± 0.007	0.26 ± 0.008	0.32 ± 0.011	0.44 ± 0.014	0.60 ± 0.021		0.711 ± 0.152	2.78 ± 0.55
							t*	2.62	1.46
							P	< .05	> .05

* Paired *t*-test, two-tailed.

comparisons. In mice killed 18 to 20 hours after either termination of fighting (11 paired comparisons) or ECS (9 paired comparisons), the apparent K_m for NE uptake was the same as for controls. Regression lines for fighting mice and controls were virtually superimposed. We do not yet know how rapidly the return to control values occurred.

Initial rates of active NE transport into synaptosomes probably are not affected by differences in the concentration of free endogenous NE within the synaptosomes that may be created by the treatments, since the kinetic constants for high-affinity NE uptake are not altered by depleting synaptosomes of NE with reserpine (*5*).

Interpreted in terms of Michaelis-Menten kinetics (*6*), our results demonstrate that a single brief episode of intense nervous stimulation, either the natural social stress of fighting or ECS, causes an immediate but temporary decrease in neuronal membrane affinity for the reuptake of neurotransmitter NE into nerve terminals of the cerebral cortex. To our knowledge, this is the first demonstration that membrane affinity for NE is acutely modulated by neuronal activity. Such a decrease in neuronal membrane affinity for NE during intense nervous stimulation has adaptive value in that it should facilitate alerting during acute emergencies and stress.

While acute nervous stimulation caused an overall decrease in net high-affinity uptake at all concentrations of [^{3}H]NE tested, we have shown that chronic fighting increased both apparent K_m and V_{max} (*2*). This increase in V_{max}, which presumably represents an adaptive increase in the total number of NE uptake sites, led to an increase in the actual net uptake at the various NE concentrations tested despite a lowered affinity for NE reuptake after chronic fighting (*2*). Thierry *et al.* (*7*) reported in vivo studies involving the stress of electric shock to the feet in rats; their results are consistent with our in vitro findings. They found that acute stress decreased the 30-minute accumulation of intracisternally administered [^{3}H]NE into the brainstem mesencephalon, but that after 3 days of such daily stress the 30-minute uptake of exogenously administered NE was increased.

Available evidence, therefore, suggests that the uptake of NE into nerve terminals in the cerebral cortex is regulated by nervous stimulation in two ways: (i) membrane affinity is acutely modulated and (ii) both membrane affinity and the number of uptake sites undergo long-term adaptive change.

Routes of investigation that may be profitable to pursue in attempting to elucidate the underlying mechanisms for these changes are suggested by a report of Maas and Mednieks (*8*) that incubating brain slices with hydrocortisone increases NE uptake, and by a report of Escueta and Appel (*9*) that a series of once daily ECS's causes sustained changes in the concentrations of synaptosomal sodium and potassium ions.

If repeated episodes of ECS should also, like repeated fighting (*2*), be shown to cause sustained adaptive changes in the kinetics of NE uptake, this might provide a plausible explanation for the mode of action of ECS, which is the most effective treatment available for elevating mood in patients suffering from mental depression (*10*). It would also provide support for the hypothesis that this common affective disorder is associated with a relative deficiency of NE at receptor sites in the brain (*11*). Data demonstrating that ECS once daily for 14 consecutive days has such effects in the cerebral cortex of mice has been obtained (*12*).

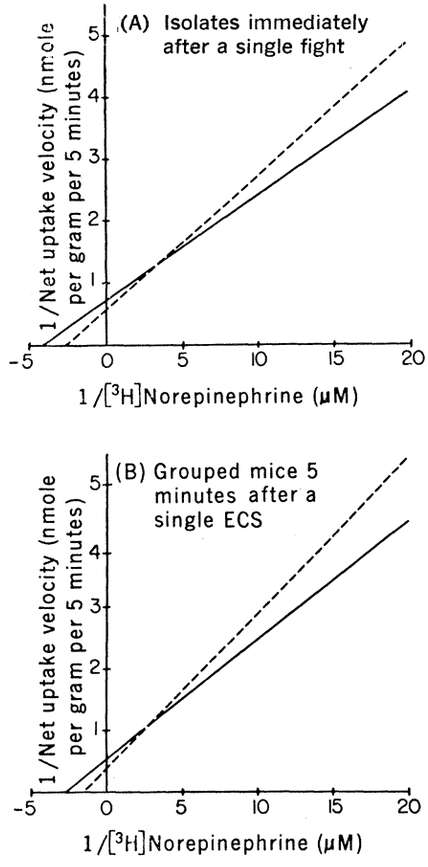

Fig. 1. Lineweaver-Burk analysis of changes in *dl*-[^{3}H]NE uptake kinetics induced by acute stimulation in male mice. The net uptake velocity (velocity at 37°C less velocity at 0°C) was determined in 5 minutes in synaptosome-rich cerebral cortical homogenates from stimulated mice (broken lines) and control mice (solid lines). Plotted are the average least squares regression lines calculated for individual mice. (A) Mice made aggressive by prolonged isolation were stimulated to fight by placing them into a group of eight to ten mice for 15 minutes, and were killed immediately thereafter. (B) Grouped mice were given a single maximal ECS and killed 5 minutes later.

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