BRAIN STEM NOREPINEPHRINE: BEHAVIOURAL AND BIOCHEMICAL DIFFERENTIATION OF RESPONSES TO FOOTSHOCK IN RATS

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VARIOUS procedures falling under the general category of "stress" have demonstrated effects on brain norepinephrine content and metabolism (see BARCHAS *et al.*, 1972). Dynamic measures of cerebral catecholamine function generally reveal that norepinephrine utilisation is facilitated in stressed subjects; conversely, drugs that reduce the levels of stored catecholamines in brain generally result in a behavioural depression. These responses have been interpreted as an attempt by the animal to maintain neurochemical homeostasis, and have been applied to various aspects of behavioural dysfunction in humans (KETY *et al.*, 1967; SCHILDKRAUT and KETY, 1967). Bidirectional mutability is at least implied by the term homeostasis. However, only two isolated instances of depressed norepinephrine utilisation after behavioural manipulation have been described (WELCH and WELCH, 1969; STONE, 1970). Additionally, there have been few instances where norepinephrine responses have been intimately related to any given behaviour (STEIN and WISE, 1969). The present report will describe experiments suggesting (a) that brain stem norepinephrine metabolism undergoes rapid, bidirectional changes, and (b) that these biochemical changes may be related to a specific constellation of behavioural responses.

We have studied norepinephrine turnover and metabolism in the brain stem of male Long–Evans rats subjected to electric footshock either with or without another rat. Shock in the presence of another rat results in the reliable appearance of aggressive responses, whereas shock alone causes prominent escape attempts. Details of the shock regimen, and of the shock-elicited fighting paradigm may be found in STOLK *et al.* (1971). To study norepinephrine turnover and metabolism, we have employed intracisternal injections of ^{3}H-dopamine, a procedure which circumvents many of the methodological difficulties associated with the cerebroventricular administration of radioactive norepinephrine itself (details of the labelled dopamine procedure are contained in STOLK, 1973). All animals in the study received the dopamine intracisternally 4 hr before commencing with behavioural manipulation; since the rats were behaviourally naïve at the time of the injection, it was assumed that all subjects were identical up to the start of behavioural manipulation. Control rats remained in their home cages. Rats receiving footshock without a partner (referred to as Shock rats) were placed into the shock chamber 4 hr after the dopamine injection. Rats shocked with another rat (referred to as Fighting rats) were placed into the chamber at the same time after the dopamine injection, and were subjected to the same sequence of shock. In all cases, the duration of footshock was 5 min. Animals were sacrificed at various times after receiving shock, and brain stem tissue was analysed at various times after receiving shock, and brain stem tissue was analysed for levels of

radioactive norepinephrine (formed from the ^{3}H-dopamine) and normetanephrine.

Immediately after rats were removed from the shock chamber, significant alterations in ^{3}H-norepinephrine levels were found only in Shock subjects (18 per cent reduction in labelled norepinephrine content compared to either Fighting or Control groups: $P < 0{\cdot}05$). Parenthetically, other brain regions failed to show any significant alterations at this time, regardless of which group was studied. Thus, Shock, but not Fighting, groups reveal a marked increase in the rate of norepinephrine turnover in brain stem during the period of shock presentation. This change was accompanied by a profound decrease in the levels of ^{3}H-normetanephrine (Table 1); again, despite exposure to identical shock parameters, Shock rats biochemically were markedly different from Fighting rats.

TABLE 1. ^{3}H-NORMETANEPHRINE LEVELS IN BRAIN STEM FOLLOWING EXPOSURE OF SHOCK RATS TO 5 MINUTES OF ELECTRIC FOOTSHOCK. [Absolute radioactivity and per cent of Control group normetanephrine levels in two individual experiments are compared at the time rats were removed from the shock chamber. Changes in normetanephrine radioactivity are contrasted with altered ^{3}H-norepinephrine levels obtained in the same rats. Values represent the means (± S.E.M.) obtained in groups of from 6 to 12 rats each. An asterisk (*) denotes a significant difference from the indicated control value. A double asterisk (**) denotes a significant difference from the ^{3}H-norepinephrine content measured in the same group.

Exp. No.	Group	^{3}H-Normetanephrine content		^{3}H-Norepinephrine % of control
		(dis/min per g)	% of control	
1†	Control	43,900 ± 6800	100 ± 14	100 ± 5
	Shock	27,200 ± 1500 (*)	62 ± 4 (*) (**)	86 ± 4 (*)
2	Control	8350 ± 700	100 ± 9	100 ± 4
	Shock	5100 ± 500 (*)	61 ± 8 (*) (**)	79 ± 9 (*)

† 3·1 μCi ^{3}H-dopamine injected in Experiment 1; 0·8 nCi were injected in Experiment 2. The dopamine specific activity in both experiments was 8·8 Ci/mmole.

Turnover of brain stem norepinephrine was estimated over the one hour period after Shock and Fighting rats were removed from the shock chamber. In contrast to the lack of change in Fighting rats observed immediately following shock presentation, norepinephrine turnover over the subsequent hour was increased substantially (Table 2). Conversely, ^{3}H-norepinephrine turnover in brain stem of Shock rats, which increased during the shock period itself (see previous paragraph), was significantly slower than both Control and Fighting group norepinephrine turnover (Table 2). These results clearly differentiate the two groups exposed to electric footshock, whether on a biochemical or a behavioural basis, and suggest that brain stem norepinephrine containing neurons participate actively in the behavioural responses evoked by electric footshock.

TABLE 2. FRACTIONAL RATE CONSTANTS (k, IN RECIPROCAL HOURS) FOR THE RATE OF DECLINE IN BRAIN STEM OF CONTROL, SHOCK AND FIGHTING GROUPS. [Rate constants were determined by least square regression analysis following logarhythmic transformation of the norepinephrine radioactivity levels obtained at selected times during the one hour period following footshock. Values represent k ± standard deviation. An asterisk (*) denotes a significant difference from all other groups.]

	Control group	Shock group	Fighting group
k:	0·373 ± 0·065 ($N = 32$)	0·246 ± 0·105 ($N = 31$)*	0·616 ± 0·104 ($N = 32$)*

Further evidence both for the increased norepinephrine turnover rate in Fighting rats and for the behavioural–biochemical relationship between shock-elicited fighting behaviour and norepinephrine metabolism is present in Fig. 1. These data demonstrate that the number of fighting episodes measured for each subject correlates extremely well with the levels of ^{3}H-norepinephrine isolated in brain stem one hour after the fighting pairs were removed from the shock chamber.

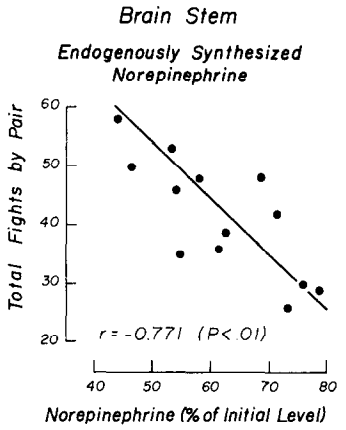

FIG.1.—Relationship between brain stem ^{3}H-norepinephrine content and magnitude of fighting behaviour in rats. Catecholamine determinations were made 1 hr after the fighting period; radioactive norepinaphrine content is expressed as per cent of level obtained prior to beginning of behavioural manipulation.

Apart from the behavioural relationships to brain stem norepinephrine metabolism documented in the studies presented above, we have also observed a significant differentiation of normetanephrine production and norepinephrine turnover. Thus, ^{3}H-normetanephrine levels are markedly reduced at a time when ^{3}H-norepinephrine turnover is increased (immediately following shock; Table 1). There are several ways to interpret this finding. One interpretation would place the results in the context of generally accepted current models of central nervous system noradrenergic function, which predict that increased release of norepinephrine into the synaptic cleft manifests itself in increased rates of normetanephrine formation (since catechol-0-methyltransferase is localised predominantly extraneuronally). By such models, these data could be taken as an indication that norepinephrine release decreases both during and after shock, regardless of whether catecholamine turnover increases or falls.

On the other hand, several other investigators also were unable to document normetanephrine accumulation during periods of increased norepinephrine utilisation following shock (STEIN and WISE, 1969; TAYLOR and LAVERTY, 1969; BLISS *et al.*, 1968). Thus, an alternate interpretation of the findings in the latter references as well as in the present study is that changes in normetanephrine alone may not be an indicator of transynaptic utilisation. This possibility is further strengthened by the fact that the model, from which the usual interpretation of transsynaptic utilisation of norepinephrine involving normetanephrine is based, is derived primarily from pharmacological studies, and information thus obtained may not be representative of

natural noradrenergic function. Hypothetically, increased utilisation may be manifest by increases in intracellular norepinephrine metabolism involving a neuroregulatory role of norepinephrine, as well as by the more popular neurotransmitter role. (We do not mean to imply that the standard model does not apply to natural function in transsynaptic transmission, since normetanephrine accumulation during accelerated turnover of brain stem norepinephrine following shock in the Fighting groups is increased, as also indicated by the data of THIERRY *et al.*, (1968); however, we raise the question of whether this is the only valid interpretation of norepinephrine's role in the brain.) The relative importance of these two alternatives in behavioural states remains to be elucidated.

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