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Central Serotonin Involvement in the Elaboration of the Startle Reaction in Rats[1]

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FECHTER, L. D. *Central serotonin involvement in the elaboration of the startle reaction in rats.* PHARMAC. BIOCHEM. BEHAV. 2(2) 161–171, 1974. – The present investigation provides evidence for serotonergic involvment in the inhibition of the acoustic startle reaction which results from the presentation of neutral stimuli (prepulses) shortly before reflex elicitation. While the specific serotonin (5-HT) synthesis inhibitor p-Chlorophenylalanine did not affect the response, a large amine-depleting dose of reserpine enhanced the reaction elicited under stable (control) stimulus conditions (no prepulse delivered). Selective replacement of 5-HT by administration of the *l*-amino acid precursor and a monoamine oxidase inhibitor further enhanced control startle amplitude and also eliminated prepulse inhibition. Chemical assays indicated especially large increases in 5-HT levels in the cortex and brainstem. Administration of the 5-HT precursor in the non-reserpinized rat both increased control startle levels and also interfered with prepulse inhibition although not as completely as in the reserpinized animal. The results indicate that 5-HT has a facilitatory effect on the reflex and suggest that a catecholaminergic system is also involved in determining reflex amplitude.

Serotonin Acoustic startle reaction Monoamines

THE AMPLITUDE of the startle reaction in the rat to a loud, sudden tone stimulus is highly dependent upon variability in the sensory context against which it is elicited. Both ambient noise level as well as the presentation of discrete, but low intensity neutral stimuli prior to reflex evocation [24,25], markedly alter the test behavior. Such findings are not unique among studies of reflex behavior [10, 23, 26, 36] and, indeed, are likely to reflect rather basic excitatory and inhibitory control over motor output along spinal reflex pathways. Moreover, the reliability with which the amplitude of the startle reaction can be altered when rather simple stimulus parameters are varied and the accumulating evidence [21,25] indicating that such changes reflect, in particular, central inhibitory control, suggests that the startle reaction is a valuable test reflex for elucidating the physiological basis of such inhibition at a behavioral level.

The present work focussed on possible neurochemical (specifically indoleamine) correlates of behavioral findings such as those described by Ison and Hammond [25]. The evidence suggesting such an investigation is considerable. There is close proximity between serotonin (5-HT) terminals in the spinal cord and α-motoneurons subserving the large flexor and extensor muscle groups [15] and altered excitability in reflex pathways has been demonstrated following modification of amine systems by pharmacological [3, 12, 13] and electrophysiological [12,31] methods. The most direct evidence for serotonergic involvement in spinal reflex behavior, however, comes from recent study of the startle reaction which suggest that habituation to a repetitive startle stimulus might be altered by administration of the 5-HT synthesis inhibitor, *p*-Chlorophenylalanine (pCPA) [1, 14, 32].

GENERAL METHODS

Animals

Male albino rats obtained from the Holtzman Rat Co., Madison, Wisconsin were used. All animals were housed individually and maintained under ad lib access to food and water and under diurnal lighting conditions (12 hour light–dark cycle).

Behavioral testing and all drug administrations were restricted to the light-on period and were begun only after the animal had been housed in the colony room for at least

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one week. At that time the animals' weights ranged from 330–430 g.

Apparatus

The apparatus used in the determination of the startle reaction has previously been described [25]. Briefly, the animal was placed in a perforated Plexiglas box (20.3 x 6.4 x 8.9 cm high) held semi-rigidily within a metal superstructure by compression springs. The startle reaction, detected by an accelerometer mounted at one end of the chamber, was fed into a Beckman dynograph recorder where it was expressed as mm of pen deflection. The test chamber was housed in a sound attenuated room with ambient noise level set at 70 dB (re: .0002 dyn/cm^2) and ambient illumination of 6.5 lx.

The startle stimulus consisted of a 10 KHz tone delivered at an intensity of 120 dB for 20 msec duration. The stimulus rise and decay time was 5 msec. Discrete white noise stimuli (prepulses) of 20 msec duration and 93 dB intensity could also be delivered prior to the startle stimulus.

Behavioral Procedure

The animal was placed in the test chamber and allowed a 5 min adaptation period prior to the beginning of the test session. Unless otherwise noted, the animal then received 49 trials: seven control trials in which the startle stimulus was presented alone and 42 prepulse trials in which a 93 dB, 20 msec white noise stimulus occurred 20, 40, 60, 80, 160, or 320 msec prior to the onset of the startle stimulus. Trials were presented on the average at 45 sec intervals (range 30–60 sec). The order of trial presentation was determined by Latin squares which were different for each animal.

Drug Preparation and Administration

All drugs were obtained in powder form and were placed in solution shortly before i.p. administration. With the exception of reserpine and pCPA, all drugs were dissolved in 0.9% saline to which a few drops of 1 N HCl were added if necessary. Reserpine was dissolved in a few drops of glacial acetic acid and distilled water; pCPA was suspended in a mixture of saline to which was added a drop of tween 80. When necessary, solutions were neutralized with sodium bicarbonate. The following drugs employed in this investigation were generously provided by Dr. C. A. Stone; Merck, Sharp and Dohme (MK-486), Dr. A. O. Geiszler; Abbot Laboratories (pargyline), and Dr. A. Weissman; Pfizer (pCPA).

Dissections and Assays

Chemical assays for noradrenaline (NA), dopamine (DA), serotonin (5-HT), and 5-hydroxy-indoleacetic acid (5-HIAA), the major metabolite of 5-HT, were performed on the cortex, brainstem, diencephalon, and striatum of individual animals randomly selected prior to behavioral testing. Fifteen min after the completion of such testing, the animal was killed by spinal concussion, the brain rapidly removed, cleansed in chilled 0.9% saline, blotted dry, dissected, and frozen at -90° C.

For analysis, the amines were extracted in 0.4 N perchloric acid to which had been added 0.2 ml 10% disodium ethylenediamine tetra acetate (EDTA) and 0.1 ml 5% sodium metabisulfite. The samples were homogenized thoroughly and then centrifuged. Separation of the amines and metabolite was achieved using Dowex 50W-X4 columns according to the method outlined by Atack and Magnusson [7] and described by Lindqvist [30].

All compounds were assayed spectrofluorometrically; NA according to Bertler, Carlsson, and Rosengren [9]; 5-HT and 5-HIAA according to Atack and Lindqvist [6] using a modification of the ophtaldialdehyde (OPT) condensation procedure [29]; DA according to a modification of the dihydroxyindole procedure [5].

The results of the assays are presented in terms of nanograms (ng) of substance per brain region. For the experiments reported here the mean weights of brain sections assayed were: cortex, 0.74 g ± 0.09; brainstem 0.41 g ± 0.05; diencephalon 0.37 g ± 0.09; and striatum 0.15 g ± 0.06.

EXPERIMENT 1: SELECTIVE SEROTONIN DEPLETION AND THE STARTLE REACTION

Procedure

The animals (N = 4) received two behavioral test sessions each consisting of 10 control trials and 40 prepulse trials – 10 each at 40, 80, 160, and 320 msec. The first session occurred three days after an injection of saline (3 ml/kg). The day after testing, all animals were injected with pCPA (320 mg/kg) and 72 hr later at the time of maximal 5-HT depletion [28] the startle reaction was again assessed.

Results

The data for the control sessions (see Fig. 1) are consistent with earlier reports [17,25] demonstrating maximal inhibition of the startle reaction on prepulse trials having an ISI of 40 msec and some return toward control levels at longer ISI's. An analysis of variance performed on the data indicated that the ISI effect was significant ($F = 21.88$, $p<0.001$). pCPA, however, did not alter the pattern of prepulse inhibition or the amplitude of the startle reaction in a stable environment in any consistent fashion ($F = 0.78$). Furthermore, no effect of pCPA was noted when dose levels as high as 900 mg/kg of pCPA (N = 12) were administered over three days, nor was there any observable effect of pCPA (320 mg/kg) on other behavioral measurements such as speed of habituation or dishabituation to a repetitive control startle stimulus (N = 18) (unpublished data). In some instances (N = 18), whole brain 5-HT levels were determined in individual animals according to the method of Welsh and Welsh [35] providing evidence that pCPA did depress the level of 5-HT. Hence, 5-HT depletion did not alter the startle reaction in any way, a result inconsistent with the hypothesis that pCPA either "releases behavioral inhibition" [14] or produces a general "hyperreactivity" [11].

EXPERIMENT 2: THE EFFECTS OF RESERPINE ON STARTLE BEHAVIOR

In this aspect of the present study, amine depletion was accomplished by administration of a single large dose of reserpine. Although this compound does not produce specific 5-HT depletion, it does offer two important advantages in the context of this investigation; firstly, it

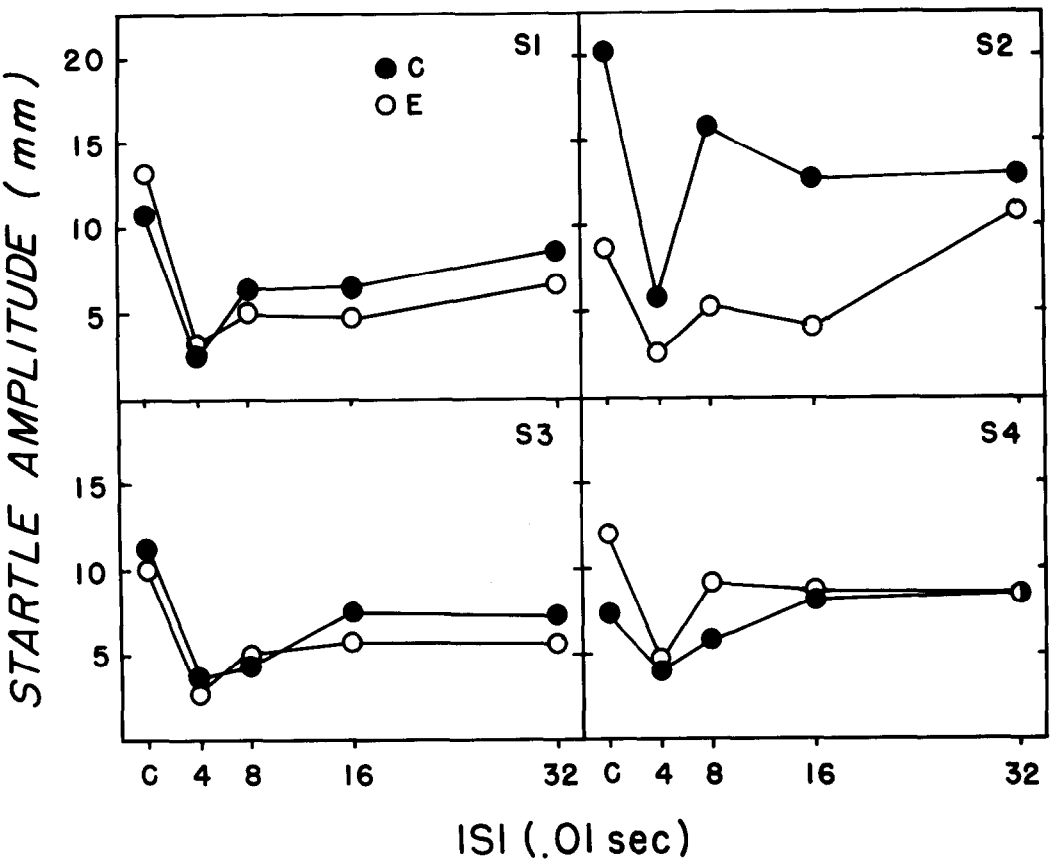

FIG. 1. Mean response amplitude for individual animals injected according to the following schedule: Saline – 3 days – Test 1 (C – filled circles) 1 day – pCPA – 3 days – Test 2 (E – open circles).

provides a means of studying the effects, on the startle reaction, of amine depletion accomplished by a method other than synthesis inhibition, and, secondly, it provides a basic pharmacological model for subsequent experiments in which amine levels can be selectively replaced by administration of the *L*-amino acid precursor.

Procedure

Animals (N = 12) were randomly assigned to groups which received either reserpine (5 mg/kg) or the neutralized acetic acid–distilled water vehicle alone. The drug concentration was 5 mg/ml. All injections were given within 3 hr of the onset of the daylight cycle.

Six hr after injection, the animals received a series of control and prepulse startle trials as outlined in the general methods. The brains of four control and three experimental animals were removed after testing to verify the drug's central action.

Results

Inspection of Fig. 2 demonstrates that reserpine enhanced the response to a control startle stimulus while not altering the pattern of preliminary-stimulus induced inhibition. While consistent enhancement of startle behavior is apparent in reserpinized animals on control and prepulse trials alike, the ratio of response amplitude at 40 msec to the respective group control level yielded essentially the same values (0.44 for control animals, 0.46 for experimental animals). It thus seems inappropriate to interpret the enhancement on prepulse trials as a lessening of prepulse inhibition.

An analysis of variance confirmed that the differences found between drug groups ($F = 12.77$ $p<0.01$) and ISI's ($F = 31.65$, $p<0.001$) were reliable and that the drug x ISI interaction effect was not significant ($F = 1.20$).

The results of the chemical assays indicate that reserpine did, in fact, reduce monoamine levels, although the extent of depletion varied in different brain regions. Serotonin levels (see Fig. 3) were markedly depressed in the cortex, brainstem, and diencephalon, areas rich in 5-HT terminals [18], while no marked change occurred in the striatal section. The 5-HT metabolite, 5-HIAA, remained at approximately normal values in the brain as well.

In order to estimate activity in the indoleamine system, a ratio of metabolite to amine content in each brain region was calculated for each animal (see Table 1). In both groups, 5-HT activity as reflected by this measure was greatest in the brainstem, at intermediate levels in the cortex and diencephalon, and lowest in the striatum. Far higher rations were noted in experimental animals, and this response is undoubtedly related to the prevention of 5-HT retention by reserpine with consequent enhancement of amine metabolism.

The catecholamine (CA) levels were also determined

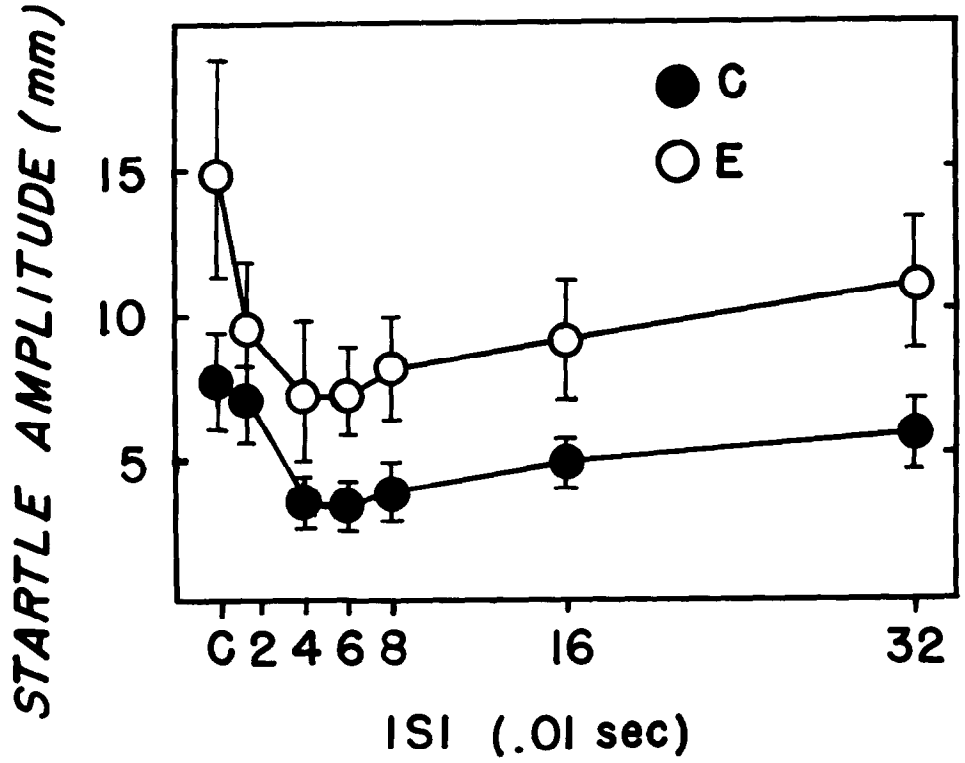

FIG. 2. Mean response amplitude and S.E.M. on control and prepulse trials in animals injected according to the following schedule: Saline – 6 hr – Test (C – filled circles); Reserpine – 6 hr – Test (E – open circles).

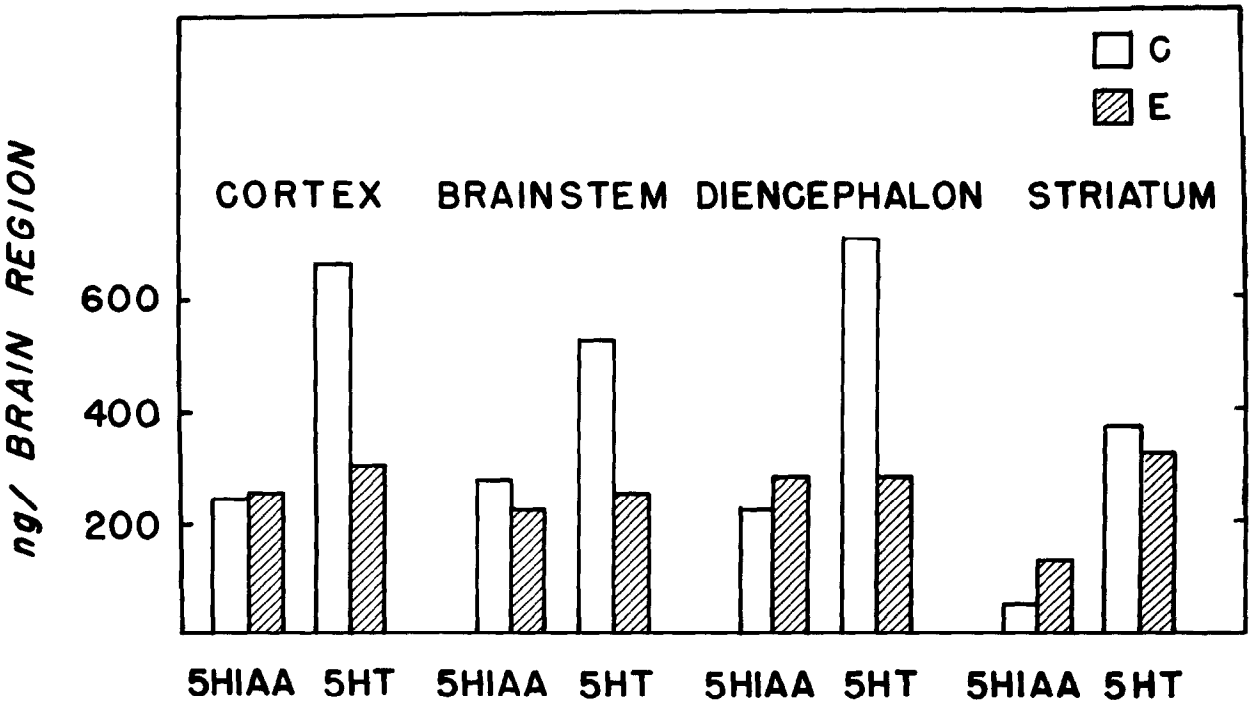

FIG. 3. Median 5-HT and 5-HIAA content of brain regions in animals injected with saline (open bars) or 5 mg/kg reserpine (hatched bars) 7 hr prior to brain dissection. Amines measured in nanograms (ng).

TABLE 1

RATIO OF 5-HIAA/5-HT IN FOUR BRAIN REGIONS OF SUBJECTS INJECTED WITH RESERPINE (5 MG/KG) OR SALINE SEVEN HOURS PRIOR TO BRAIN DISSECTION

Cortex	Brainstem	Diencephalon	Striatum
Saline group			
0.44	0.69	0.42	0.26
0.31	0.42	0.33	0.20
0.34	0.52	0.24	0.10
0.33	0.48	0.32	0.09
$\bar{X}$ = 0.36	0.53	0.33	0.16
Reserpine group			
0.89	1.07	1.20	0.14
1.43	2.10	0.83	0.57
0.66	0.82	1.01	0.40
$\bar{X}$ = 0.99	1.33	1.01	0.37

with marked reduction of NA noted in all areas with appreciable NA-containing terminals (see Fig. 4).

Reserpine administration markedly reduced DA levels in diencephalon and striatal sections and produced a smaller decline in the cortex. Although appreciable DA concentrations remained in the brainstem and cortex following reserpine, it is unlikely that this indicates continuing DA-based neurotransmission since few, if any, DA terminals have been found in these regions [34].

In summary, the results of this experiment provide general support for the thesis that the monoamines are involved in the acoustic startle reaction. Reserpine treatment is capable of altering control over the reaction such that responses elicited in stable sensory environments are elevated. The data, too, provide additional evidence for partial dissociation between the mechanisms involved in determining the level of the startle reaction in control and prepulse trials for although enhanced responsivity was evident in the former condition, the pattern of prepulse inhibition was not noticeably altered by reserpine treatment.

EXPERIMENT 3: REPLACEMENT OF 5-HT IN THE RESERPINIZED SUBJECT

The previous experiment demonstrated that general monoamine depletion resulted in the enhancement of the control startle reaction, but that it did not alter the pattern of prepulse inhibition. In this experiment, 5-HT was selectively increased in reserpine-treated animals by the administration of the precursor, 5-hydroxytryptophan (5-HTP).

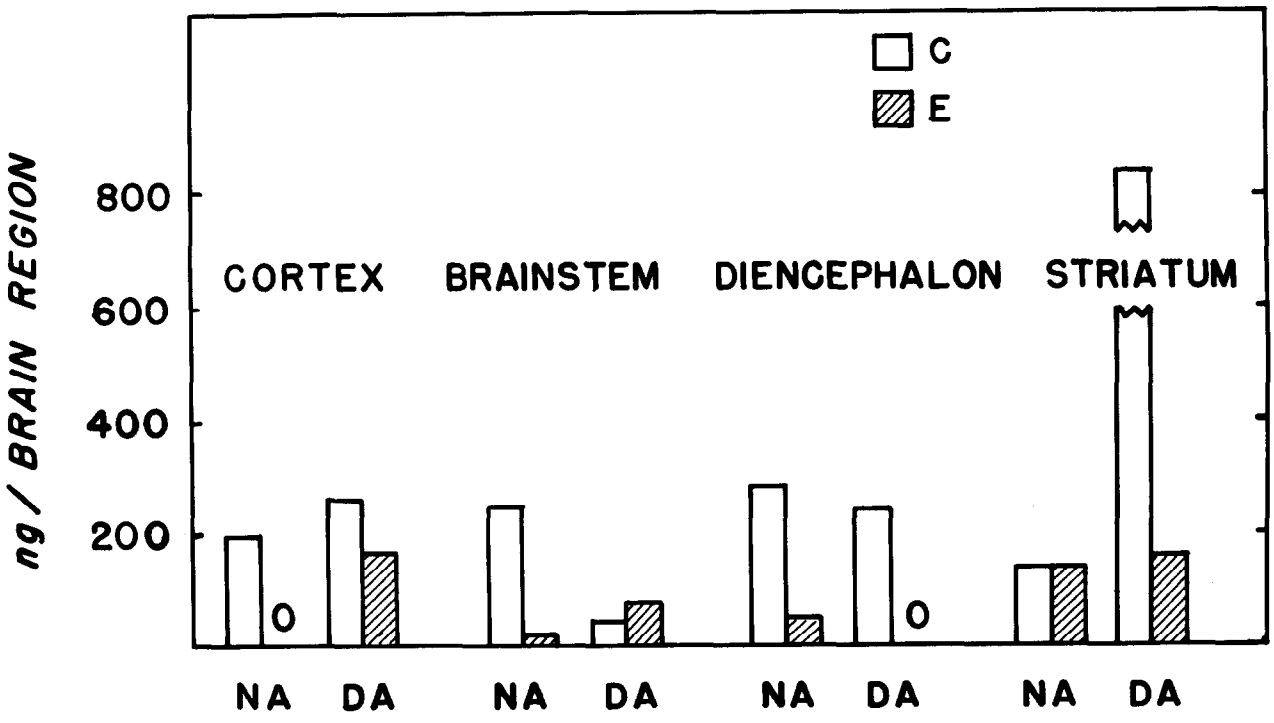

FIG. 4. Median NA and DA content of brain regions in animals injected with saline (open bars) or 5 mg/kg reserpine (hatched bars) 7 hr prior to brain dissection. Amines measured in nanograms (ng).

Procedure

The animals (N = 12) were injected with reserpine (5 mg/kg) soon after the onset of the daylight cycle, and then, 6 hr later, received the peripheral decarboxylase inhibitor l-α-methyl-α-hydrozino-β-3,4 dihydroxyphenyl-proprionic acid, levo form (MK-486, 100 mg/kg). This substance has been shown to block peripheral utilization of 5-HTP and to restrict 5-HT accumulations to brain regions containing endogenous indole stores [8]. Animals randomly assigned to the experimental group were then given the monoamine oxidase (MAO) inhibitor pargyline (75 mg/kg) 6 1/2 hr after reserpine and 5-HTP (30 mg/kg) 7 hr after reserpine; control animals received pargyline and saline (rather than 5-HTP) according to the same time schedule. Injection concentrations were 5 mg/ml for reserpine, 10 mg/ml for MK-486, 25 mg/ml for pargyline, and 10 mg/ml for 5-HTP. All animals received the standard behavioral test session consisting of 7 control trials and 42 prepulse trials. The brains of four control and three experimental animals were analyzed by chemical assays.

Results

Administration of 5-HTP after reserpine produced marked enhancement of startle levels relative to the control group (see Fig. 5) and essentially prevented prepulse inhibition. The reliability of this finding is supported by statistical analyses (of trends) which show that the shape of the functions obtained from the two groups differed significantly (vis: linear $F = 10.83$, $p<0.01$ and quadratic $F = 72.710$, $p<0.0000$ components of variance and the drug x ISI quadratic interaction $F = 16.83$, $p<0.005$ were statistically significant).

As anticipated, 5-HT levels were markedly higher among experimental animals than among control animals not receiving the 5-HT precursor (see Fig. 6, note change in scale) and, indeed, were above levels determined in saline treated animals (see Fig. 3). There was also a slight increase in 5-HIAA levels in the cortex, brainstem, and diencephalon (but not the striatum) of animals receiving the precursor. This finding probably reflects competition between very high levels of 5-HT and pargyline for MAO and also suggests regional differences in 5-HT metabolism. Regional differences were also noted in the amount of 5-HT accumulated in the control animals receiving the MAO inhibitor but not the precursor, 5-HTP. Serotonin accumulated most in the brainstem and cortex, with much less accumulation noted in the diencephalon and striatum.

CA levels were reduced to blank levels in all regions in both experimental and control animals (see Fig. 7, note change in scale) thereby rendering any interpretation of

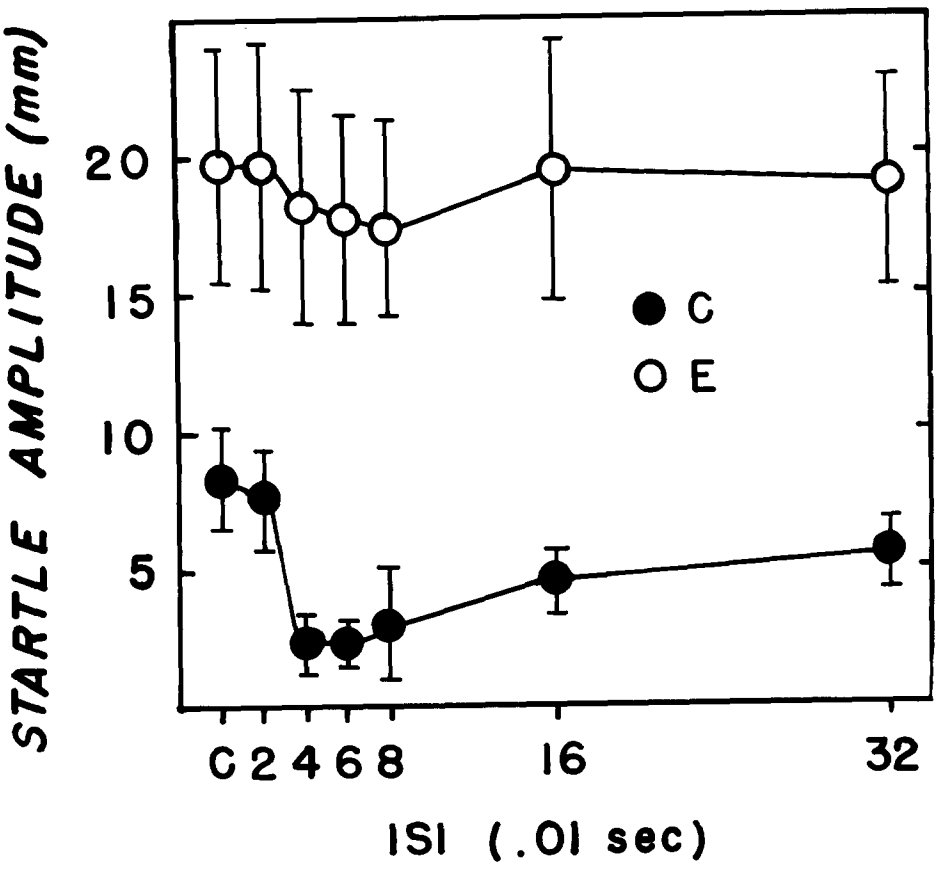

FIG. 5. Mean response amplitude and S.E.M. on control (C) and prepulse trials in animals injected according to the following schedule: Control group (C – filled circles); Reserpine – 6 hr – MK-486 – 30 min – Pargyline – 30 min – Saline – 40 min – Test. Experimental group (E – open circles); Reserpine – 6 hr – MK-486 – 30 min – Pargyline – 30 min – 5-HTP – 40 min – Test.

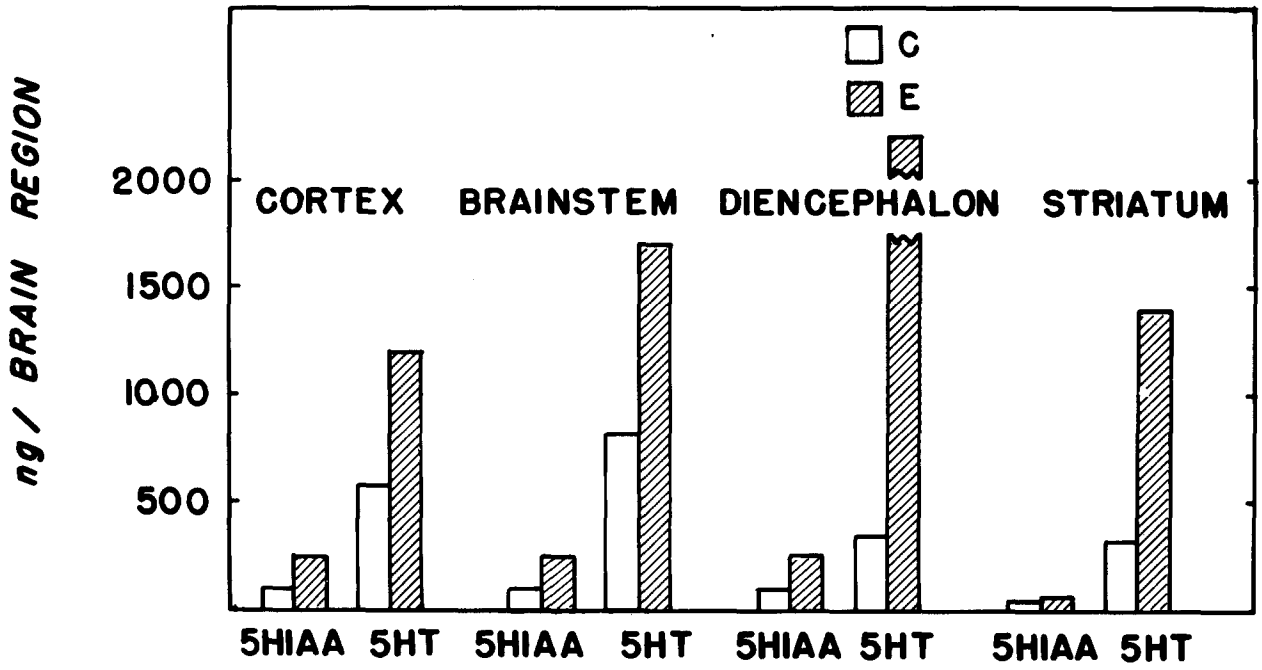

FIG. 6. Median 5-HT and 5-HIAA content of brain regions in animals injected with 5mg/kg reserpine, 100 mg/kg MK-486, 75 mg/kg pargyline, and 30 mg/kg 5-HTP (E – hatched bars) or control animals injected with saline instead of 5-HTP (C – open bars). Amines measured in nanograms (ng).

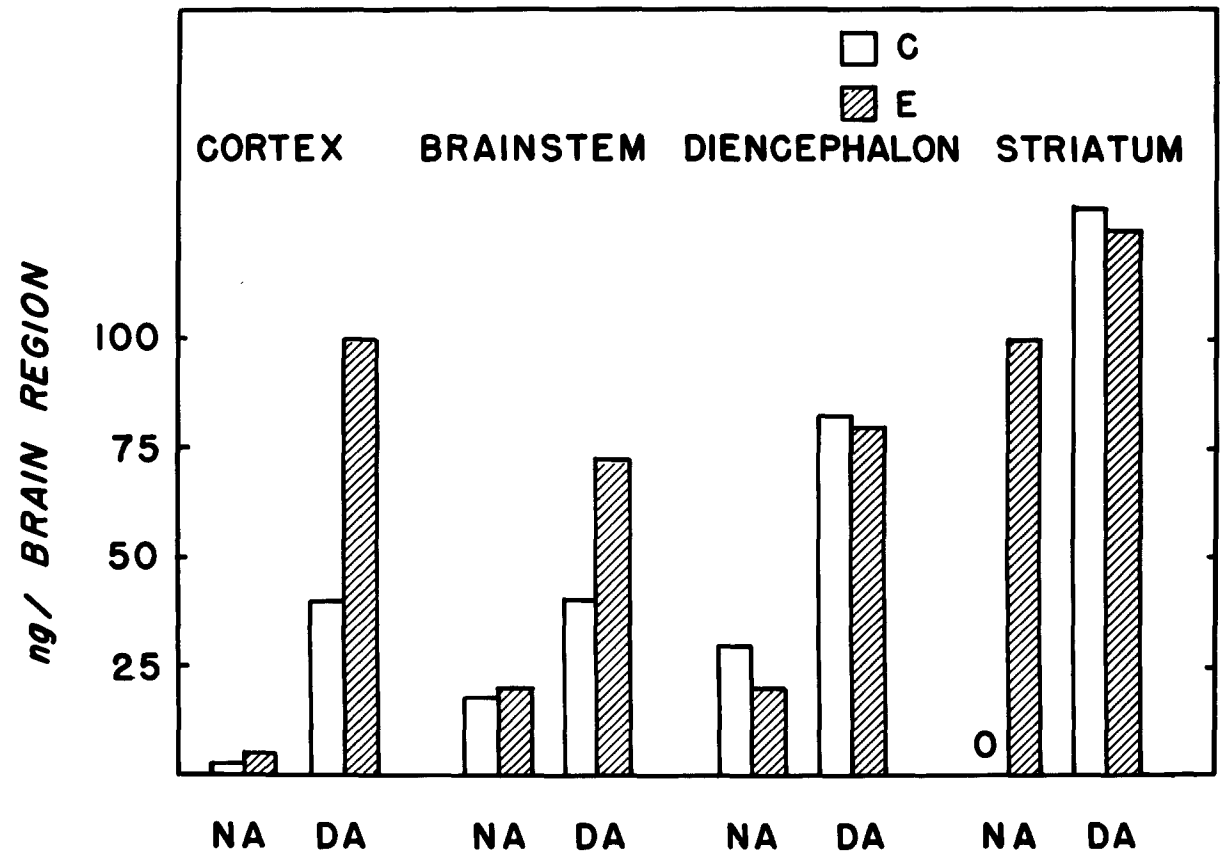

FIG. 7. Median NA and DA content of brain regions in animals injected with 5 mg/kg reserpine, 100 mg/kg MK-486, 75 mg/kg pargyline, and 30 mg/kg 5-HTP (E – hatched bars) or control animals injected with saline instead of 5-HTP (C – open bars). Amines measured in nanograms (ng).

differences in CA content between groups unreliable. Even the two most substantial differences between groups, the increase in DA in the cortex and of NA in the striatum of experimental animals, are unlikely to reflect functional differences between the groups since the CA terminals in these regions are principally noradrenergic and dopaminergic respectively.

Hence, treatment of reserpine-treated animals with 5-HTP produced markedly higher control startle levels than those observed following reserpine-induced amine depletion and prevented prepulse inhibition. Analysis of 5-HT levels in the brain suggests that these behavioral effects result from enhancement of 5-HT levels in certain brain regions.

This study would indicate that the enhancement of the startle response in a stable sensory environment noted following reserpine treatment alone was not elicited by the depletion of 5-HT. Since, indeed, 5-HT appears to exert an enhancing effect upon the startle reaction in both stable and non-stable sensory environments, the reserpine induced response would appear to reflect interference with another chemical system.

EXPERIMENT 4: EFFECTS OF ENHANCING SEROTONIN LEVELS IN THE NON-RESERPINIZED ANIMAL

Because CA levels were also markedly reduced in the previous study and because other experiments [16] suggested that NA activity is associated with depression of the startle reaction elicited in stable sensory environments, the current investigation was designed to determine whether the enhancement in startle reactivity noted after reserpine and 5-HTP would be manifested in animals with intact CA systems.

Procedure

The animals (N = 12) were randomly assigned to two groups which received 100 mg/kg MK-486 followed 15 min later by 75 mg/kg pargyline. One-half hr later, an injection of 5-HTP (30 mg/kg) or saline, depending on group assignment, was given. Each animal was tested 40 min after the last injection. The brains of three animals from each group were dissected for chemical assays.

Results

The results portrayed in Fig. 8 demonstrate an enhancement of startle reactivity on control trials and slight prepulse inhibition among those animals which received 5-HTP. Prepulse inhibition, though markedly reduced from that observed either in saline or reserpine treated animals of the previous experiments appears to be a reliable effect. While the differences in response level between drug groups ($F = 32.54$, $p<0.0005$) and ISI's ($F = 7.97$, $p<0.0000$) were

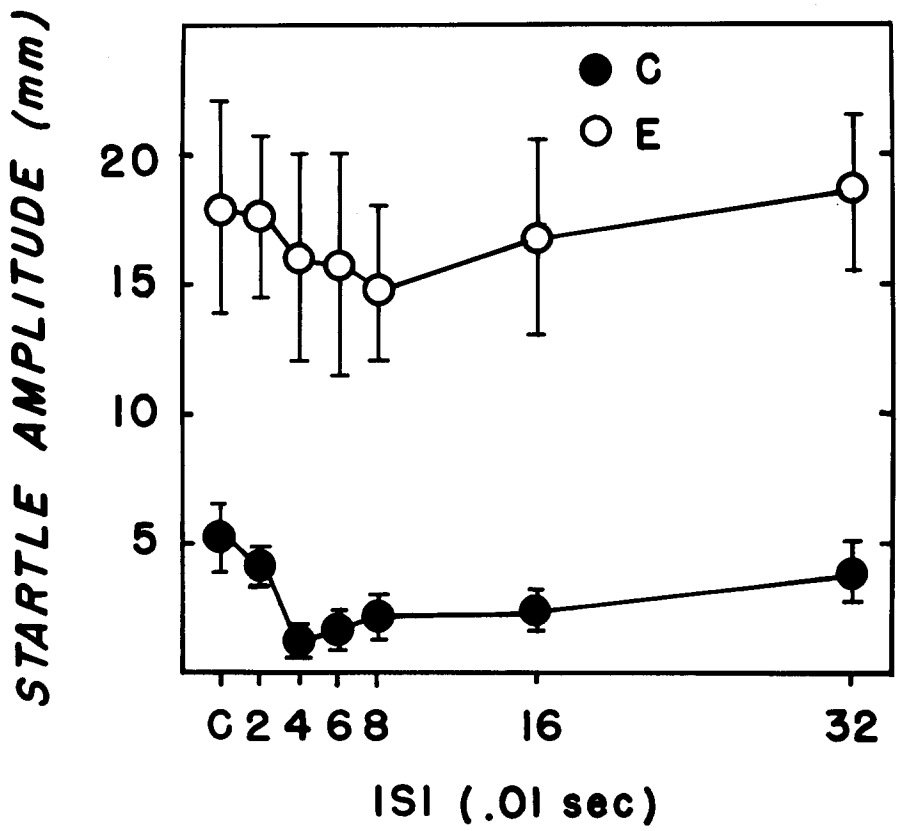

FIG. 8. Mean response amplitude and S.E.M. on control (C) and prepulse trials in animals injected according to the following schedule: Control group (C – filled circles); MK-486 – 15 min – Pargyline – 30 min – Saline – 40 min – Test. Experimental group (E – open circles); MK-486 – 15 min – Pargyline – 30 min – 5-HTP – 40 min – Test.

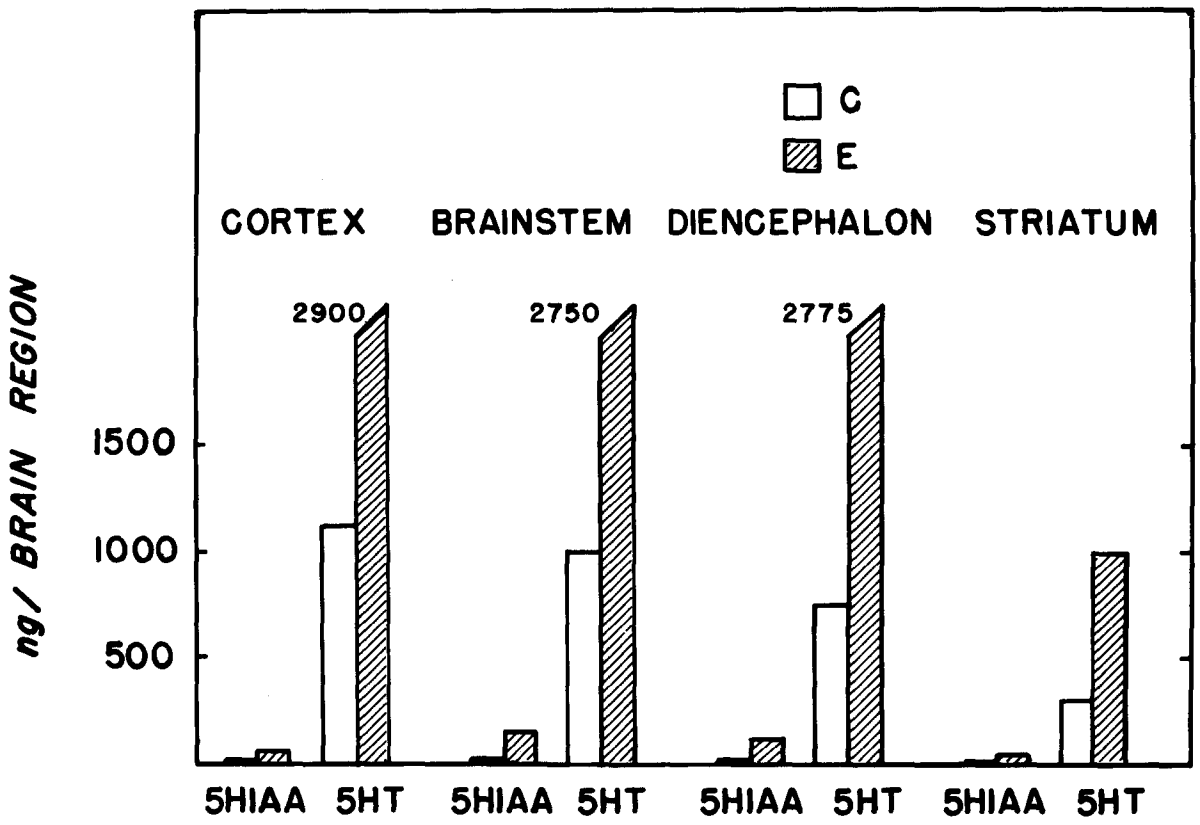

FIG. 9. Median 5-HT and 5-HIAA content of brain regions in animals injected with 100 mg/kg MK-486, 75 mg/kg pargyline, and 30 mg/kg 5-HTP (E – hatched bars) or control animals injected with saline instead of 5-HTP (C – open bars). Amines measured in nanograms (ng).

significant there was no evidence of a reliable drug x ISI interaction ($F = 1.14$).

The chemical assays (see Fig. 9) indicate very high levels of 5-HT in cortex, brainstem, and diencephalon with noticeably lower levels in the striatum of experimental animals, an effect corresponding to the distribution of endogenous amine. Serotonin levels were also noticeably higher among these experimental animals than in those having been pretreated with reserpine.

Assays for NA and DA (see Fig. 10) indicated some depression in cortex and brainstem regions of experimental animals as compared to animals receiving MK-486, pargyline, and saline. This change might reflect either enhanced activity or some CA displacement by 5-HT. The marked differences in DA content in the striatum cannot be interpreted since subsequent information indicated the presence of a spurious contaminant in the assay procedure.

These data confirm that elevating 5-HT in the brain does enhance startle reactivity and suggest that the CA's might be capable of depressing the startle response on prepulse trials.

GENERAL DISCUSSION

The present investigation has directly demonstrated the involvement of 5-HT in the elaboration of the acoustic startle reaction in rats and strongly suggests the existence of a CA system having at least partially antagonistic effects on the behavioral level. The results indicate that enhancement of 5-HT levels by administration of the precursor, 5-HTP, and an MAO inhibitor, pargyline, produced a marked increase in the amplitude of the reaction elicited on control trials and a decrease in the degree of response inhibition normally produced by prepulse stimuli. The latter effect was even more evident when 5-HT levels were increased above normal in animals which had been pretreated with an amine-depleting dose of reserpine.

The data suggest that 5-HT exerts a facilitatory influence on motor output measured behaviorally, and, in this regard, are consistent with evidence that the administration of the 5-HT precursors, tryptophan and 5-HTP, enhance spinal reflex behavior at behavioral [4] and electrophysiological [2] levels in acute spinal cats. The observation that enhancement of brain 5-HT levels prevented prepulse inhibition is congruent with Aghajanian and Sheard's [1] demonstration of renewed responsivity to a previously habituated click or air-puff startle stimulus by caudal rephe stimulation. While habituation to sensory stimuli and stimulus-induced inhibition of the sort used in this report may represent different processes, it is intriguing that pharmacological stimulation of 5-HT receptors and electrical stimulation of brainstem regions containing 5-HT cell bodies both reversed stimulus-induced depression of the same motor response.

It was demonstrated, too, that reserpine treatment alone, which interfered with monoamine storage and pro-

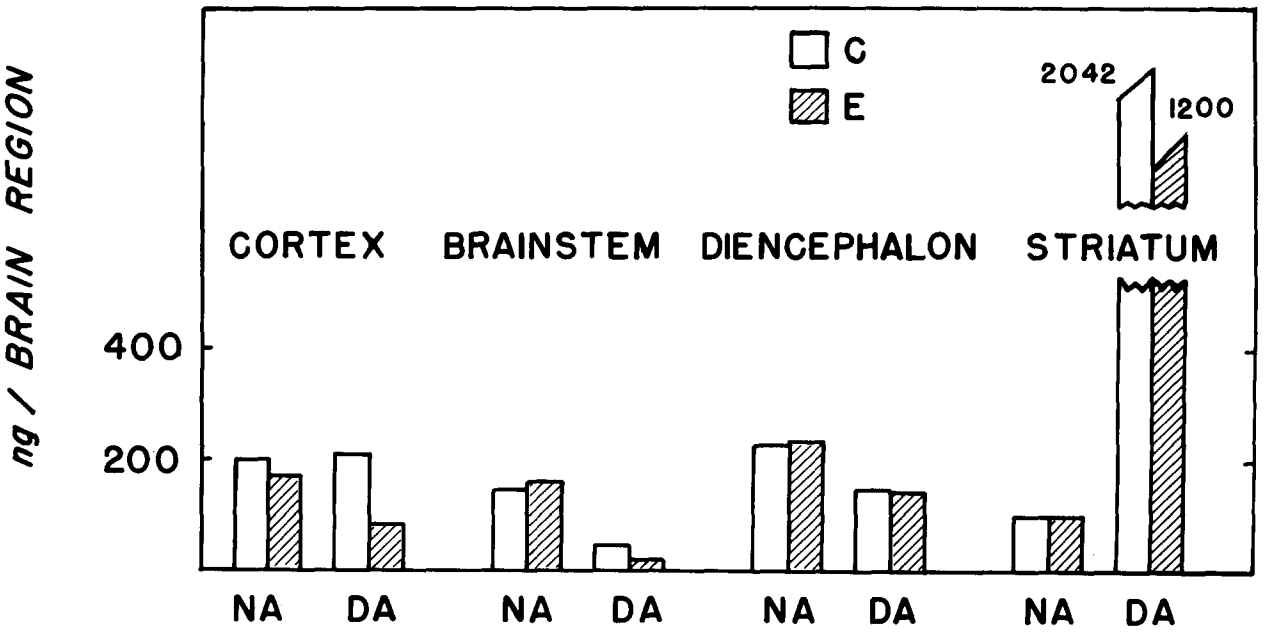

FIG. 10. Median NA and DA content of brain regions in animals injected with 100 mg/kg MK-486, 75 mg/kg pargyline, and 30 mg/kg 5-HTP (E – hatched bars) or control animals injected with saline instead of 5-HTP (C – open bars). Amines measured in nanograms (ng).

duced general monoamine depletion, enhanced the startle reaction on control trials. Since pCPA did not produce similar effects, it seems likely that the reserpine effects observed reflected interference with CA neurotransmission. It is of further interest, that reserpine, while exerting no effect by itself on prepulse inhibition, potentiated the blocking effect of 5-HT on such inhibition. One possible interpretation of this finding is that a CA system normally exerts an inhibitory effect upon 5-HT neurons which are, themselves, excitatory to the test behavior.

The use of systemically administered drugs does not allow a discrete analysis of the neurochemical pathways involved in the control of prepulse startle reaction. Neither is it possible to identify with complete certainty the loci where drug-induced alterations of receptor activity interdigitate with such pathways. Yet the results of chemical analyses of brain regions, showing only modest changes in amine levels in the diencephalon and striatum and marked changes in cortex and brainstem, are consistent with investigations into the effects of surgical lesions upon the startle reaction in the rat. Kemble and Ison [27] were unable to disrupt the startle reaction elicited in stable or non-stable stimulus environments following destruction of structures in the limbic system; Hammond and Thomas [20] reported no difference in control startle amplitude between control animals and rats which received septal lesions and were tested after the hyperreactive septal rage syndrome had disappeared. Hammond [19], however, did demonstrate that the startle reaction elicited under stable stimulus conditions could be enhanced by lesions in the medial frontal cortex and that the same response could be degredated by lesions placed in n. reticularis pontis caudalis. Szabo and Hazafi [33] found similar effects in cats after lesions in n. reticularis pontis gigantocellularis and rostral portions of n. regicularis pontis caudalis. The failure of ablation techniques to disrupt prepulse inhibition is somewhat less surprising since lesions are most likely to depress amine levels [22], and this study suggests that it is enhancement of 5-HT receptor activity which alters the behavioral response.

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