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DIFFERENTIAL EFFECTS OF ANTIPSYCHOTIC DRUGS ON THE NEUROTENSIN CONCENTRATION OF DISCRETE RAT BRAIN NUCLEI

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Abstract—The present study mapped the topographic distribution of, and the effect of neuropharmacologically distinct antipsychotic drugs on, the concentration of neurotensin (NT) in the rat brain at the level of discrete nuclei or areas. The chronic administration of either haloperidol or clozapine increased the concentration of NT-like immunoreactivity (NT-LI) in the nucleus accumbens and decreased it in the medial prefrontal and cingulate cortex and in the interstitial (bed) nucleus of the stria terminalis. In contrast, the prolonged administration of haloperidol, but not clozapine, increased the concentration of NT-LI in the anterior caudate nucleus and posterior caudate-putamen. The concentration of NT-LI in the great majority of the rat brain nuclei examined was unaffected by the chronic administration of either antipsychotic drug. This pattern of pharmacological response distinguishes NT from all other neuropeptides which have been shown to be influenced by prolonged antipsychotic drug administration. These findings suggest that the functional information imparted to NT-containing cells by neuronal dopamine (DA) release, as inferred from the consequences of receptor blockade, varies remarkably between different populations of DA neurons and further implicate NT as a neuroanatomically-selective neurochemical substrate of the adaptive responses mediating the therapeutic and motoric side effects of antipsychotic drugs.

Schizophrenia is increasingly recognized to be a major, world-wide public health problem in need of more aggressive and innovative research. The putative neurotransmitter dopamine (DA‖) is the most implicated neurochemical substrate of the pathogenesis and pharmacotherapy of schizophrenia [1–3], though these two roles are inequivalently supported by empirical evidence. Little evidence has accumulated in support of a DA defect in schizophrenia [4, 5] though broad-based pharmacological data implicate brain DA neurons in the actions of antipsychotic drugs [6–10]. Thus, DA-containing neurons may not be etiologically involved but rather a critical neuronal subset or population may represent a compensatory mechanism and site of antipsychotic drug action.

While the introduction of antipsychotic drugs into psychiatric practice represents one of the great advances in psychopharmacology, accumulated experience indicates that the available agents are imperfect solutions having major, sometimes crippling, side effects and are not effective in all patients. The elucidation of distal co-substrates of drug action which express the consequences of antipsychotic drugs on DA neurons would appear to be of paramount importance to the design of safer and more effective pharmacotherapeutic agents and to advancing our understanding of the neurochemical pathology of schizophrenia. One such potential co-substrate is neurotensin (NT), a tridecapeptide which is well represented and unevenly distributed in the central nervous system (CNS) of mammals [11, 12] and fulfills a number of criteria defining a neurotransmitter or neuromodulator role. Accumulating evidence indicates that the alteration of DA neuronal interactions with NT-containing neurons may be crucial to antipsychotic drug action. First, NT is a well documented DA neuronal population-selective affector and effector [13–16]. Anatomical and functional evidence indicate that NT receptors are localized on DA cell bodies and terminals [14, 15, 17, 18] and that DA neurons regulate the density of NT receptors in the CNS [15]. Second, the intracerebroventricular administration of NT elicits a number of behavioral, physiological, neurochemical and electrophysiological effects strikingly reminiscent of the preclinical pharmacology of antipsychotic drugs [19]. Third, similar to the gradually developing, non-tolerating course of their clinical antipsychotic activity [20, 21], the chronic administration of antipsychotic drugs to rats results in an initially submaximal, sustained increase in the NT content of DA-rich brain regions [22, 23]. While the involvement of NT as a pathogenic factor and/or co-substrate of antipsychotic drug action has not been directly demonstrated in clinical studies, the observation of a normalization of the subnormal CSF

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‖ Abbreviations: DA, dopamine; NT, neurotensin; NT-LI, neurotensin-like immunoreactivity; and VTA, ventral tegmental area.

concentrations of NT in a subgroup of drug-free schizophrenics with a course of antipsychotic drug treatment [24] supports this association.

In an attempt to investigate further the relevance of antipsychotic drug-induced alterations in brain regional NT content to their clinical antipsychotic properties, we sought to expand upon these initial preclinical studies [22, 23] in two ways. First, would the incorporation of a greater degree of anatomical resolution of drug effects on neuronal NT content yield increasingly complex patterns of effects reflective of the heterogeneity of the association of brain DA and NT systems? Second, is the differential preclinical and clinical neuropharmacology of different antipsychotic drugs expressed on the synthesis and/or utilization of NT as estimated by peptide content? Both haloperidol and clozapine have been demonstrated to be effective in the treatment of the symptoms of schizophrenia but differ in other aspects of their pharmacology, most notably a differential liability to induce extrapyramidal side effects [25], and are considered to represent prototypal typical and atypical antipsychotics respectively. Their antipsychotic and extrapyramidal side effects are proposed to reflect selective drug actions on the mesolimbocortical and nigrostriatal DA neuronal systems respectively [1, 2, 26, 27]. A general working hypothesis of this laboratory is that these DA systems, particularly the mesolimbocortical, are actually composed of functionally distinct DA neuronal populations (subsystems) which may be delineated by pharmacological response. Therefore, in these experiments the chronic administration of antipsychotic drugs was used both as a neurobiological probe to delineate the DA neuronal population-selective consequences of prolonged DA receptor blockade on NT mechanisms and as a means of further defining the neuroanatomical and neurochemical substrates of their therapeutic and extrapyramidal side effects. The results obtained indicate four distinct patterns of drug effect and suggest that the functional influence of DA neurons on NT-containing cells differs remarkably between different brain regions and that DA–NT interactions in selective neuronal populations may be involved in the therapeutic or motoric side effects of antipsychotic drugs.

METHODS

General. Male Sprague–Dawley albino rats (Charles Rivers, Wilmington, MA) weighing 214–275 g upon initiation of drug or vehicle administration were used as experimental subjects. Animals were housed in pairs in temperature- and humidity-controlled quarters maintained on a 12-hr light/dark cycle with lights on at 7:00 a.m. and free access to food and water. Groups of rats (N = 6–16 per group) received daily (10:00 a.m. to noon) intraperitoneal (i.p.) injections of either haloperidol (1 mg/kg, McNeil Laboratories), clozapine (20 mg/kg, Sandoz Pharmaceuticals) or vehicle (0.3% tartaric acid, 1 ml/kg) for 14 consecutive days. The site of injection was alternated on a daily basis between the left and right peritoneum, and body weights were recorded on alternate days. Animals were decapitated 24 hr following the last injection and the brains were rapidly removed, frozen on dry ice, and stored at −75° until dissected. This dosing regimen and last dose-sacrifice interval were selected based upon the finding of a maximal haloperidol-induced increase in NT content in the nucleus accumbens in rats killed 24 hr following 2 weeks of daily administration of 1 mg haloperidol/kg body weight [22].

Microdissection of discrete brain nuclei or areas. Discrete brain nuclei or areas were micropunch-dissected from unfixed, frozen coronal brain sections [28, 29] as previously described [30]. Brains were mounted using a glycol matrix in a cryostat/microtome (Damon/IEC, Needham Heights, MA) and sliced into 300 μm thick coronal sections at a chamber temperature of −10°. Brain nuclei or areas were bilaterally (except the midline raphe nuclei) micropunched from consecutive slide-mounted sections using thin-walled stainless steel tubing of 0.57, 0.75, 0.91 or 1.15 mm i.d. with the aid of a stereomicroscope. The atlas of Konig and Klippel [31] and the reports of Palkovits *et al.* [32] and Jacobowitz and Palkovits [33] served as general guides for dissection. Samples were blown into 500 μl polypropylene microcentrifuge tubes, frozen on dry ice, and stored at −75° until assayed.

Quantification of NT content by radioimmunoassay. Samples were homogenized by ultrasonic disruption in 500 μl of ice-cold 1 M HCl and centrifuged (10,000 *g* for 10 min at 4°), and duplicate 200 μl aliquots of the resulting supernatant fraction were frozen, lyophilized, and assayed for NT-like immunoreactivity (NT-LI) by a previously described double antibody equilibrium radioimmunoassay (RIA) [34]. Tissue pellets were assayed for protein content by a phenol/Folin reagent method [35] using bovine serum albumin as a standard. The antibody used recognizes the mid-portion (Arg-Pro-Lys, amino acids 6, 7 and 8) of the NT molecule and, when used at a final antiserum dilution of 1:10,000, can reliably detect 1.25 pg of NT per assay tube. Recovery of synthetic NT added to the supernatant fraction averaged 85–90%. Reference standards (1.25 to 5120 pg) of synthetic NT and lyophilized tissue extracts were reconstituted in 200 μl of assay buffer (0.01 M sodium phosphate, 0.15 M sodium chloride, 0.25 M EDTA, 0.1% sodium azide, 0.1% gelatin, pH 7.6). Antiserum was diluted in assay buffer containing 1.5% normal rabbit serum, and 100 μl was added to each assay tube. Radioactive tracer prepared by a chloramine T catalyzed iodination of NT with $Na^{125}I$ was purified by carboxymethylcellulose column chromatography, and 20,000 cpm of trace in 50 μl of assay buffer was added to all assay tubes. Tubes were vortexed and incubated for 24 hr at 4°. Following incubation, 5 μl of goat-antirabbit serum was added to the assay tubes. Tubes were vortexed and incubated for an additional 24 hr at 4° after which the tubes were centrifuged and the supernatant fractions aspirated. Radioactivity in the pellets was determined using an LKB 1274 gamma counter with a 2 min/tube counting time and a 67% counting efficiency. All samples were assayed in a single assay with a sensitivity of 2.5 pg and an IC_{50} of 80 pg with a 5% intra-assay variability between duplicate samples. Results

are expressed in pg of NT-LI per milligram of tissue protein and were uncorrected for recovery.

Statistics. Effects of antipsychotic drugs on the concentration of NT-LI in discrete brain nuclei or areas were analyzed by a Student–Keuls multiple range test or Scheffe's test for multiple group comparisons following a one-way analysis of variance. Significant statistical differences were defined by a probability level of 0.05.

RESULTS

While both the haloperidol- and drug vehicle-treated groups exhibited comparable weight gain over the course of the dosing regimen studied, those animals receiving clozapine demonstrated an initial decrease (for 3–4 days) in weight gain followed by a similar rate of gain relative to the other two experimental groups (Fig. 1). As a result, the body weights of the clozapine-treated animals at the termination of the study were significantly less than those of subjects receiving haloperidol or vehicle.

Examination of the topographic distribution of NT-LI in the brains of vehicle-treated animals (Table 1) indicated that the concentration of NT-LI varied greatly both between different brain structures and between the component nuclei of certain structures (e.g. amygdala, hypothalamus). Values obtained in chronically vehicle-treated animals did not differ from corresponding values from uninjected control subjects (data not shown). The predominant localization of NT in nuclei or areas of subcortical limbic structures and the midbrain and medulla parallels that defined by immunohistochemical techniques [36] and RIA using a carboxy terminus [11] or an amino terminus [12] directed antibody. The highest concentrations of NT-LI was found in the central amygdaloid nucleus and the interstitial (bed) nucleus of the stria terminalis. The remaining amygdaloid nuclei and the septal nuclei contained intermediate concentrations of NT-LI. In a separate experiment, an examination of the relative concentration of NT-LI in the functionally and neurochemically delineated dorsal and ventral subdivision of the bed nucleus of the stria terminalis indicated a preferential and dense localization of NT-LI in the ventral (3792 ± 430 pg/mg protein, mean ± SE, N = 7) versus the dorsal (2045 ± 216 pg/mg protein, N = 7) subdivision. The hypothalamus was rich in NT-LI, though the distribution between the component nuclei of the hypothalamus was more uniform than that of the amygdala. The hippocampus, as well as other limbic and nonlimbic cortex, contained relatively low amounts of NT-LI. In the ventromedial mesencephalon, NT-LI was well represented and preferentially localized in the nuclear groups of the ventral tegmental area (VTA) relative to groups comprising the substantia nigra. Other midbrain nuclei representing the major cell bodies of origin for the monoamine neurotransmitters (i.e. dorsal and medial raphe, locus coeruleus) similarly contained relatively high concentrations of NT-LI.

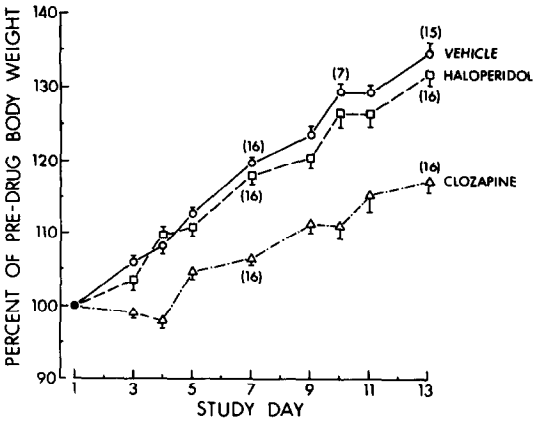

Fig. 1. Effect of the repeated administration of haloperidol, clozapine or the drug vehicle on the body weight of groups (N = 8 unless otherwise indicated in parentheses) of rats expressed as a percent (mean ± SEM) of the pre-drug (study day 1) value. See text for details of dosing regimens. Rats weighed 214–275 g upon initiation of drug or vehicle administration.

The effects of the chronic administration of haloperidol or clozapine on the concentration of NT-LI in discrete brain nuclei or areas are summarized in Table 1 and Fig. 2. The NT-LI content of the great majority of the brain nuclei or regions examined was unaffected by the chronic administration of either antipsychotic drug. In agreement with a previous report [22], the prolonged administration of haloperidol produced a significant increase in the NT-LI content of the nucleus accumbens and caudate nucleus. The chronic administration of clozapine similarly increased the concentration of NT-LI in the nucleus accumbens. However, unlike haloperidol, repeated clozapine administration failed to alter the NT-LI content of the caudate nucleus. This dissociation of response to haloperidol and clozapine was observed in both the anterodorsal caudate nucleus (A 8620–A 7890 μm*) and the posterior caudate-putamen (A 4890–A 4110 μm*). In striking contrast to these antipsychotic drug-induced increases, both clozapine and haloperidol significantly decreased the concentration of NT-LI in the medial prefrontal cortex, anterior cingulate cortex and interstitial (bed) nucleus of the stria terminalis. In contrast to the drug-induced decreases noted in neocortical areas, the NT-LI content of allocortical areas (entorhinal and piriform cortex) was not affected by the repeated administration of either antipsychotic drug. Of the ventral mesencephalic nuclear groups examined, the concentration of NT-LI was affected significantly by clozapine, but not haloperidol, only in the DA cell body rich pars compacta of the substantia nigra. Further differential effects of the two antipsychotic drugs were noted in some limbic nuclei where clozapine and haloperidol increased the concentration of NT-LI in the cortical amygdaloid nucleus and the medial preoptic nucleus, respectively, and in the brainstem where clozapine increased the concentration of NT-LI in the periaqueductal grey.

* Anterior-posterior coordinates from Konig and Klippel [31].

Table 1. Effect of the chronic administration of haloperidol or clozapine on the concentration of neurotensin-like immunoreactivity (NT-LI) in discrete rat brain nuclei or areas

	NT-LI (pg/mg protein)		
		Treatment	
Brain nuclei or area	Vehicle	Haloperidol	Clozapine
Amygdaloid			
Central	3060 ± 227	2997 ± 171	3159 ± 215
Lateral	790 ± 63	683 ± 40	1096 ± 177
Basal	878 ± 78	863 ± 73	774 ± 35
Medial	757 ± 40	711 ± 30	692 ± 31
Cortical	573 ± 35	556 ± 36	771 ± 99*
Posterior	359 ± 31	344 ± 33	436 ± 30
Medial posterior	413 ± 20	430 ± 24	447 ± 31
Basal posterior	540 ± 26	620 ± 41	657 ± 39
Septal			
Lateral	893 ± 57	918 ± 32	999 ± 57
Medial	730 ± 61	822 ± 102	902 ± 47
Interstitial (bed) nucleus of stria terminalis	3194 ± 133	2708 ± 115*	2438 ± 169*
Hypothalamic			
Paraventricular	1831 ± 303	2020 ± 200	1963 ± 265
Periventricular	1315 ± 374	1139 ± 308	1404 ± 301
Dorsomedial	1904 ± 399	1841 ± 151	2131 ± 342
Ventromedial	688 ± 78	675 ± 44	610 ± 37
Medial preoptic	1747 ± 43	2347 ± 167*	1998 ± 106
Lateral preoptic	1792 ± 165	1668 ± 126	1561 ± 135
Arcuate	1024 ± 100	1148 ± 98	1313 ± 124
Anterior	1700 ± 288	2035 ± 147	1774 ± 97
Hippocampus			
Dorsal	120 ± 10	93 ± 14	147 ± 16
Ventral	43 ± 3	43 ± 3	48 ± 3
Nucleus accumbens	287 ± 35	521 ± 35*	414 ± 28*
Olfactory tubercle	551 ± 30	542 ± 40	504 ± 30
Cerebral cortex			
Medial prefrontal	180 ± 14	97 ± 24*	64 ± 14*
Cingulate	116 ± 8	95 ± 3*	74 ± 8*
Piriform	229 ± 53	209 ± 38	271 ± 72
Entorhinal	157 ± 12	147 ± 14	175 ± 16
Basal ganglia			
Anterodorsal caudate nucleus	61 ± 8	164 ± 12*	48 ± 5
Posterior caudate-putamen	306 ± 26	444 ± 49*	341 ± 20
Ventromedial mesencephalon			
Ventral tegmental area			
Lateral	1654 ± 146	1757 ± 136	1795 ± 397
Medial	1262 ± 120	1152 ± 173	1472 ± 137
Substantia nigra			
Pars compacta	540 ± 37	620 ± 20	738 ± 39*
Pars lateralis	474 ± 41	582 ± 43	608 ± 39
Pars reticulata	369 ± 14	369 ± 16	382 ± 21
Brainstem			
Raphe			
Dorsal	1349 ± 66	1432 ± 214	1469 ± 154
Medial	1027 ± 68	1074 ± 76	980 ± 79
Locus coeruleus	1628 ± 178	1861 ± 543	1747 ± 350
Periaqueductal grey	1424 ± 104	1708 ± 144	2299 ± 310*

Values represent the mean ± SEM of six to sixteen determinations.
* $P < 0.05$ compared to vehicle-injected control subjects.

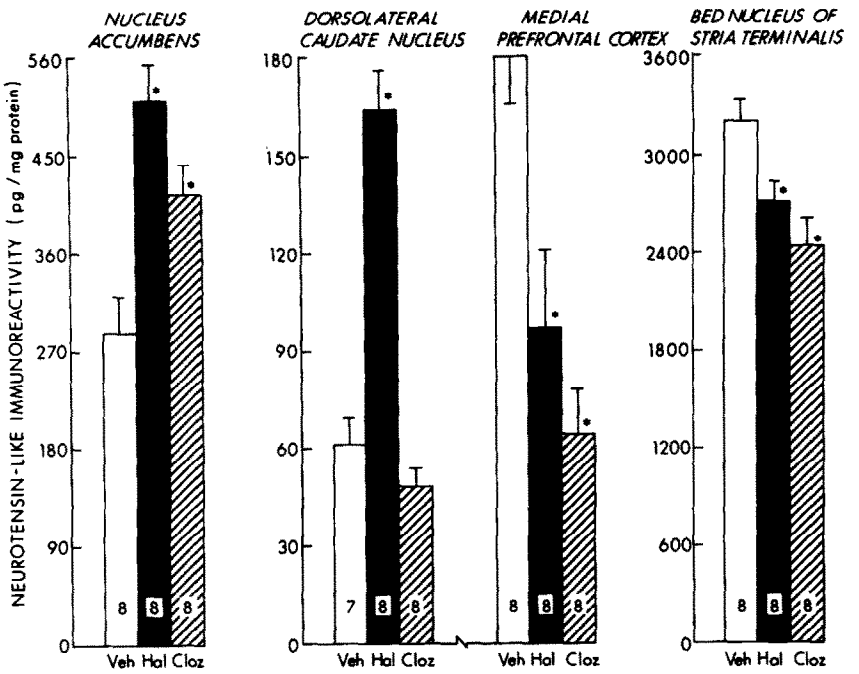

Fig. 2. Effect of the chronic administration of haloperidol, clozapine or the drug vehicle on the concentration of neurotensin-like immunoreactivity in selected brain nuclei or areas. See text for details of dosing regimens. Values are means ± SEM; N = number shown at the bottom of each column. Key: (*) $P < 0.05$ compared to vehicle-injected controls.

DISCUSSION

The intent of the present study was to evaluate further the role of NT as a co-substrate (with DA) of the pharmacology of antipsychotic drugs. Such a co-substrate would have to satisfy specific physiological and pharmacological criteria including demonstrating functionally significant, neuronal population-selective, interactions with DA neurons and be influenced by antipsychotic drugs in a non-tolerating, sustained manner consistent with a role in the adaptive neuronal mechanisms thought to mediate their therapeutic effects. The former criterion appears to be well satisfied [13, 14, 16, 37], and the latter was addressed by mapping the effect of the chronic administration of distinct antipsychotic drugs on brain NT concentration at a level of anatomical resolution of drug effect commensurate with (1) the anatomical, functional and biochemical heterogeneity of brain DA neuronal populations [38–42] and (2) the functional and anatomical organization of limbic brain structures [43]. The micropunch dissection scheme was therefore optimized for sampling the discrete component nuclei or subdivisions of the amygdala, septum, hypothalamus, bed nucleus of the stria terminalis and hippocampus and the neuronal projection fields and cell body groups of the major DA systems in the CNS. The limbic system was selected for neuroanatomic focus due to the rich neuropeptidergic innervation of limbic structures [44–46] which are thought to represent the neuroanatomical substrates of schizophrenia [47, 48].

If anatomical distribution is a relative index of the functional organization of a neurotransmitter or neuromodulator, then the uneven distribution of NT-LI would indicate an uneven influence of NT as a neurotransmitter or neuromodulator in the CNS. The remarkably high concentrations of NT-LI in the central amygdaloid nucleus and the bed nucleus of the stria terminalis are consistent with the presence of a major NT pathway projecting between these limbic brain structures via the stria terminalis [49]. The relatively high density of DA innervation of the central amygdaloid nucleus [30, 50] is strategically positioned to influence this NT pathway as well as other neuropeptides comprising the large peptidergic outflow exiting this amygdaloid nucleus [45], thus raising the possibility of a critical role for this neural circuit in antipsychotic drug action. The preferential distribution of NT-LI in the medial (i.e. nucleus interfascicularis and nucleus linearis caudalis) and lateral (i.e. nucleus parabrachialis pigmentosus and nucleus paranigralis) VTA compared to the nuclear groups of the substantia nigra is consistent with the influence of NT on the function of mesolimbocortical DA systems compared to other DA neuronal populations eminating from the ventromedial mesencephalon [13, 14]. It is of interest that the locus coeruleus and the dorsal and medial raphe nuclei also contain high concentrations of NT-LI, suggesting that a role for NT in regulating the activity of monoamine transmitter-containing neurons at the pivotal level of their cell bodies may not be unique to DA.

The prolonged administration of two pharmacologically distinct antipsychotic drugs evoked multiple, diverse patterns of effect on brain nuclei concentrations of NT-LI. These results have impor-

tant implications for our understanding of both the functional association between DA- and NT-containing cells in the CNS and the mechanisms of action of antipsychotic drugs. From a neurobiological perspective, the diversity of response of NT-containing cells to prolonged DA receptor blockade suggests that neuronal DA release encodes distinct functional information to differing populations of NT-containing cells, perhaps as a function of varying points of synaptic contact by DA neurons (e.g. axo-somatic, axo-dendritic or axo-axonic). Alternatively, it is equally plausible that the differential response of populations of NT-containing cells may reflect their differential neuroregulation by non-DA neurotransmitters (e.g. acetylcholine, norepinephrine, histamine) which are directly and/or indirectly influenced by haloperidol or clozapine. These findings reinforce and expand the concept of the pharmacological heterogeneity of DA systems terminating in different brain structures and in the component nuclei of certain structures. As pharmacological observations generally have physiological corollaries, a parcelling of DA function in the CNS into many more neuronal systems or subsystems than previously proposed based upon anatomical comparisons [38] is suggested. Consistent with our findings, we propose that the physiological, pharmacological and pathological heterogeneity of DA systems in the CNS is a function of the heterogeneity and differential localization of cells which synapse on, or are synapsed by, DA neurons and further that NT mechanisms represent a DA system-selective transducer of the differential postsynaptic response to the common event of neuronal DA release.

From a mechanism of drug action perspective, both haloperidol and clozapine altered the concentration of NT-LI in the nucleus accumbens, medial prefrontal cortex and cingulate cortex—an effect consistent with their shared properties as clinically efficacious antipsychotics and the presumed involvement of these limbic/cortical DA neuronal projection fields in mediating their therapeutic effects. However, haloperidol, but not clozapine, increased the concentration of NT-LI in the caudate nucleus, a terminal projection of the nigrostriatal DA system—a result consistent with their differential liability to induce extrapyramidal side effects and the critical involvement of this DA system in the physiology of movement. This differential pharmacology may reflect the differential ability of haloperidol and clozapine to induce striatal DA receptor supersensitivity following their repeated administration [51] or drug differences in influencing non-DA neurotransmitter systems [52]. Moreover, the differential pharmacological effects of the prolonged administration of haloperidol and clozapine on the concentration of NT-LI in the terminal projection fields of the nigrostriatal and mesoaccumbens DA systems also parallels their differential ability to induce a neuronal feedback-dependent depolarization inactivation as measured in their distinct midbrain cell bodies, (A9* and A10 respectively) [54, 55]. The interesting possibility therefore exists that the differential involvement of NT elements in the feedback regulation of nigrostriatal (A9) and mesolimbocortical (A10) DA systems may mediate or reflect (e.g. as a function of diminished neuronal DA release) antipsychotic drug-induced depolarization inactivation. The antipsychotic drug-induced alteration of the content of NT-LI in the medial prefrontal cortex, nucleus accumbens and caudate nucleus may be causally related to the increased density of [^{125}I]NT binding sites induced in these same structures by chronic treatment with pipotiazine, a DA receptor blocker [15].

In contrast to the nucleus accumbens, the chronic administration of neither haloperidol nor clozapine appreciably altered the concentration of NT-LI in the olfactory tubercle and thus argues strongly against the often-practiced, uncritical pooling of these two brain structures in the study of the "mesolimbic" DA system. The lack of effect of chronic haloperidol administration on the content of NT-LI in the DA-rich nuclear areas of the ventromedial mesencephalon does not parallel the increased density of [^{3}H]NT binding sites observed in the substantia nigra following repeated haloperidol treatment [56]. This disparity of effect on these synaptic markers may reflect the differing values of the two measures as indices of the functional state of NT-containing cells, the nerve terminal expression (altered NT content) of effects (increased density of NT binding sites) mediated in the substantia nigra, or dissimilarities in the dosing regimens used in the two studies.

The antipsychotic drug-induced decrease in the NT content of the bed nucleus of the stria terminalis suggests a pivotal, clinically relevant influence on this major gating and processing center for the output of the limbic system [46], perhaps mediated at NT cell bodies originating in the central amygdaloid nucleus [49]. A possible clinical parallel for the antipsychotic drug-induced decrease in the NT content of the medial prefrontal cortex is furnished by the reportedly greater NT concentration in the prefrontal cortex (Brodman's area 32) of post-mortem tissue from schizophrenic subjects relative to controls [57]. The significance of the differential effects of chronic haloperidol and clozapine administration on the concentration of NT-LI in certain brain nuclei or areas (i.e. cortical amygdaloid nucleus, medial preoptic area and periaqueductal grey) is not clear but may relate to the identification of these areas as particularly responsive sites for eliciting varied effects following the microinjection of exogenous NT [58, 59]. In summary, these results suggest that NT is involved in defining the functional, pharmacological and pathological inequivalence of DA systems in the CNS. While the brain regional concentrations of many neuropeptides have been shown to be altered by prolonged antipsychotic drug administration [60–66], the level of anatomical resolution employed in the present study indicates unique patterns of response for NT consistent with a role in mediating the DA system-selective effects of typical and atypical antipsychotic drugs.

These data suggest that alterations in the synthesis and/or utilization of NT represent an adaptive response to prolonged DA receptor blockade and

* Nomenclature from Dahlstrom and Fuxe [53].

further that this response is variably expressed, highly discrete, and DA neuronal population-specific. The neurobiological and pharmacological significance of these results will best be assessed using measures more predictably and directly related to the functional dynamics of NT-containing neurons, though our limited current understanding of the events of DNA transcription, mRNA translation and post-translational processing involved in the synthesis of NT has precluded the availability of molecular probes and other useful tools.

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REFERENCES

1. J. R. Stevens, *Archs gen Psychiat.* **29**, 177 (1973).
2. S. W. Matthysee, *Fedn Proc.* **32**, 200 (1973).
3. S. H. Snyder, *Am. J. Psychiat.* **133**, 197 (1976).
4. J. L. Haracz, *Schizophrenia Bull.* **8**, 438 (1982).
5. P. L. Carlton and P. Manowitz, *Neurosci. Biobehav. Rev.* **8**, 137 (1984).
6. D. S. Bell, *Br. J. Psychiat.* **111**, 701 (1965).
7. B. Angrist, G. Sathananthan, S. Wilk and S. Gershon, *J. psychiat. Rev.* **11**, 13 (1974).
8. L. L. Iversen, *Science* **188**, 1984 (1975).
9. I. Creese, D. Burt and S. H. Snyder, *Science* **192**, 481 (1976).
10. P. Seeman, T. Lee, M. Chau-Wong and K. Wong, *Nature, Lond.* **261**, 717 (1976).
11. R. M. Kobayashi, M. Brown and W. Vale, *Brain Res.* **126**, 584 (1977).
12. P. C. Emson, M. Goedert, P. Horsfield, F. Rioux and S. St. Pierre, *J. Neurochem.* **38**, 992 (1982).
13. C. B. Nemeroff, D. Luttinger, D. E. Hernandez, R. B. Mailman, G. A. Mason, S. D. Davis, E. Widerlov, G. D. Frye, C. D. Kilts, K. Beaumont, G. R. Breese and A. J. Prange, *J. Pharmac. exp. Ther.* **225**, 337 (1983).
14. P. W. Kalivas, *J. comp. Neurol.* **226**, 495 (1984).
15. D. Herve, J. P. Tassin, J. M. Studler, C. Dana, P. Kitabgi, J. P. Vincent, J. Glowinski and W. H. Rostene, *Proc. natn. Acad. Sci. U.S.A.* **83**, 6203, (1986).
16. C. D. Kilts, G. Bissette, S. T. Cain, K. M. Skoog and C. B. Nemeroff, in *Dopamine Systems and Their Regulation* (Eds. D. N. Woodruff, J. A. Poats and P. J. Roberts), p. 223. Macmillan, London (1986).
17. R. Quirion, C. C. Chiueh, H. D. Everist and A. Pert, *Brain Res.* **327**, 385 (1985).
18. J. M. Palacios and M. J. Kuhar, *Nature, Lond.* **294**, 587 (1981).
19. C. B. Nemeroff, *Biol. Psychiat.* **15**, 283 (1980).
20. B. H. Beckman, H. Hippius and E. Rutler, *Prog. Neuro-Psychopharmac.* **3**, 47 (1979).
21. T. J. Crow, *Adv. Biochem. Psychopharmac.* **24**, 495 (1980).
22. S. Govoni, J. S. Hong, H-Y. T. Yang and E. Costa, *J. Pharmac. exp. Ther.* **215**, 413 (1980).
23. M. Goedert, S. D. Iversen and P. C. Emson, *Brain Res.* **335**, 334 (1985).
24. E. Widerlov, L. H. Lindstrom, G. Besev, P. J. Manberg, C. B. Nemeroff, G. R. Breese, J. S. Kizer and A. J. Prange, *Am. J. Psychiat.* **139**, 1122 (1982).
25. J. Gerlach, P. Koppellhus, E. Hehveg and A. Munrad, *Acta psychiat. scand.* **50**, 410 (1975).
26. R. J. Baldessarini and D. Tarsy, *A. Rev. Neurosci.* **3**, 23 (1980).
27. A. Y. Meltzer and S. M. Stahl, *Schizophrenia Bull.* **2**, 19 (1976).
28. M. Palkovits, *Brain Res.* **59**, 449 (1973).
29. M. Palkovits and M. J. Brownstein, in *Brain Microdissection Techniques* (Ed. A. C. Cuello), p. 1. John Wiley, New York (1983).
30. C. D. Kilts and C. M. Anderson, *Neurochem. Int.* **9**, 437 (1986).
31. J. F. R. Konig and R. A. Klippel, *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem.* Williams & Williams, Baltimore (1963).
32. M. Paklovits, J. M. Saavedra, R. M. Kobayashi and M. Brownstein, *Brain Res.* **79**, 443 (1974).
33. D. M. Jacobowitz and M. Palkovits, *J. comp. Neurol.* **157**, 13 (1974).
34. G. Bissette, C. Richardson, J. S. Kizer and C. B. Nemeroff, *J. Neurochem.* **43**, 283 (1984).
35. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
36. L. Jennes, W. E. Stumpf and P. W. Kalivas, *J. comp. Neurol.* **210**, 211 (1982).
37. R. Quirion, *Peptides* **4**, 609 (1983).
38. O. Lindvall and A. Bjorklund, in *Clinical Neuroanatomy* (Ed. P. C. Emson), p. 229. Raven, New York (1983).
39. R. Y. Moore and F. E. Bloom, *A. Rev. Neurosci.* **1**, 129 (1979).
40. M. J. Bannon, R. L. Michaud and R. H. Roth, *Molec. Pharmac.* **19**, 270 (1981).
41. C. D. Kilts and C. M. Anderson, *Brain Res.* **416**, 402 (1987).
42. C. D. Kilts, C. M. Anderson, T. D. Ely and J. K. Nishita, *J. Neurosci.*, **7**, 3961 (1987).
43. R. L. Isaacson, *The Limbic System.* Plenum Press, New York (1982).
44. M. Palkovits, *Prog. Neurobiol.* **23**, 151 (1984).
45. G. W. Roberts, P. L. Woodhams, J. M. Polak and T. J. Crow, *Neuroscience* **7**, 99 (1982).
46. P. L. Woodhams, G. W. Roberts, J. M. Polak and T. J. Crow, *Neuroscience* **8**, 677 (1983).
47. P. Gloor, A. Olivier, L. F. Quesney, F. Anderman and S. Horowitz, *Ann. Neurol.* **12**, 129 (1982).
48. B. Bogerts, E. Meertz and R. Schonfeldt-Bansch, *Archs gen. Psychiat.* **42**, 784 (1985).
49. G. R. Uhl and S. H. Snyder, *Brain Res.* **161**, 522 (1979).
50. J. H. Fallon, D. A. Konzell and R. Y. Moore, *J. compl. Neurol.* **180**, 509 (1978).
51. N. M. Rupniak, M. D. Hall, S. Mann, S. Fleminger, G. Kilpatrick, P. Jenner and C. D. Marsden, *Biochem. Pharmac.* **34**, 2755 (1985).
52. L. Chiodo and B. S. Bunney, *J. Neurosci.* **5**, 2539 (1985).
53. A. Dahlstrom and K. Fuxe, *Acta physiol. scand.* (Suppl.) **232**, 1 (1964).
54. L. A. Chiodo and B. S. Bunney, *J. Neurosci.* **3**, 1607 (1983).
55. F. G. White and R. Y. Wang, *Science* **221**, 1054 (1983).
56. G. R. Uhl and M. J. Kuhar, *Nature, Lond.* **309**, 350 (1984).
57. C. B. Nemeroff, W. W. Youngblood, P. J. Manberg, A. J. Prange and J. S. Kizer, *Science* **221**, 972 (1983).
58. B. V. Clineschmidt, G. E. Martin and D. F. Veber, in *Neurotensin, A. Brain and Gastrointestinal Peptide* (Eds. C. B. Nemeroff and A. J. Prange), p. 283. Annals N.Y. Acad. Sci., New York (1982).
59. P. W. Kalivas, C. B. Nemeroff and A. J. Prange, in *Neurotensin, a Brain and Gastrointestinal Peptide* (Eds. C. B. Nemeroff and A. J. Prange), p. 307. Annals N.Y. Acad. Sci., New York (1982).

60. P. Frey, *Eur. J. Pharmac.* **95**, 87 (1983).
61. J. Chou, J. Tang, H-Y. T. Yang and E. Costa, *J. Pharmac. exp. ther.* **229**, 171 (1983).
62. J. S. Hong, H-Y. T. Yang, W. Fratta and E. Costa, *J. Pharmac. exp. ther.* **205**, 141 (1978).
63. M. F. Beal and J. B. Martin, *Neurosci. Lett.* **47**, 125 (1984).
64. J. S. Hong, H. A. Tilson and K. Yoshikawa, *J. Pharmac. exp. ther.* **224**, 590 (1982).
65. R. Quirion, P. Gaudreau, J-C. Martel, S. St-Pierre and N. Zamir, *Brain Res.* **331**, 358 (1985).
66. N. Lindefors, E. Brodin and U. Ungerstedt, *Neuropeptides* **7**, 265 (1986).