MECHANISMS OF ACTION OF CENTRALLY ACTING ANTIHYPERTENSIVE DRUGS

by

Parley William Madsen III

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This dissertation has been read by each member of the following supervisory committee and by majority vote has been found to be satisfactory.

Chairman: Donald N. Franz

Salvatore J. Fidone

Bradford A. Cire

Stuart A. Turkanis
To the Graduate Council of The University of Utah:

I have read the dissertation of Parley William Madsen III in its final form and have found that (1) its format, citations, and bibliographic style are consistent and acceptable; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the Supervisory Committee and is ready for submission to the Graduate School.

Date

Donald N. Franz
Chairperson, Supervisory Committee

Approved for the Major Department

Donal D J Reed
Chairman / Dean

Approved for the Graduate Council
ABSTRACT

The dose-response effects of clonidine on transmission through somatospinal reflex, viscerospinal reflex, intraspinal, and spinal-bulbospinal reflex pathways were determined in spinal or chloralose-anesthetized cats to assess principle sites of drug action. Clonidine rapidly produced parallel, dose-dependent depression of transmission through each pathway which was antagonized by tolazoline or yohimbine. The order of descending sensitivity was found to be spinal-bulbo-spinal, intraspinal, and spinal reflex pathway. Analysis of the relative depression of transmission at spinal and at brainstem levels indicates that the spinal site is more sensitive to clonidine that it is generally considered to be.

The effect of the serotonin (5-HT) precursor 5-hydroxytryptophan (5-HTP) was also assessed on the intraspinal pathway and somatospinal reflex. In contrast to clonidine, 5-HTP was more effective in depressing the spinal reflex than the intraspinal pathway, and both pathways could be depressed completely. Clonidine and 5-HTP appear to depress the excitability of sympathetic neurons by activating alpha_2- and 5-HT receptors, respectively.

The intraspinal pathway was rapidly and markedly enhanced for 1-2 hours by two methylxanthines. Clonidine depressed intraspinal transmission and prevented enhancement by the xanthines; alpha_2-receptor
antagonists blocked the effect of clonidine and not only restored but also markedly prolonged the enhancement by the xanthines. The results suggest that the excitability of sympathetic preganglionic neurons may be regulated by cyclic AMP through activation of different subtypes of adrenergic receptors that are either positively or negatively coupled to adenylate cyclase.

Methyldopa (MD) produced a moderate enhancement of transmission through three central sympathetic pathways three hours after an i.v. infusion of 150 mg/kg. However, a subsequent dose of 5 mg/kg dose of reserpine, which alone causes no depression, produces prompt, marked depression of transmission through each pathway which is antagonized by yohimbine, suggesting that reserpine releases an active metabolite of MD to depress sympathetic preganglionic neurons by activating alpha2-receptors. Depletion of epinephrine by blockade of phenylethanolamine-N-methyltransferase prevents this depression that occurs with the transmitter release.

Propranolol modestly enhances transmission through the pathways tested.
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INTRODUCTION

Essential hypertension significantly alters the life expectancy of a patient as an inverse function of the elevation in blood pressure and increases the morbidity associated with non-fatal cardiovascular events. The association of increased morbidity and mortality with hypertension was first documented by life insurance underwriters but the causal relationship had been unappreciated as clinically significant by the medical profession until the recent past (41,73,83). Although drug therapy has been demonstrated to reverse pathological changes associated with malignant hypertension for over 35 years, many health professionals cautioned against the use of antihypertensives until the results of the first long-term clinical trials were available (41,83). Until these studies documented the value of antihypertensive medications, the following quote from a monograph on hypertension published in 1944 summarizes the prevailing attitude of the medical profession regarding the therapeutic management of the hypertensive patient:

Treatment of hypertensive disease is rational only when it is directed toward the ultimate cause. Attempts to treat the disease with agents aimed solely at depressing the blood pressure level neglect the essential fact that elevated blood pressure is merely a manifestation of a fundamental fault which continues to operate despite artificial lowering of the blood pressure....
Thiocyanate serves as an example of the ineffectiveness of those therapeutic agents aimed solely at reduction of blood pressure....

There is no justification for the use of thiocyanate in hypertensive patients for the purpose of lowering blood pressure because, in common with other depressor drugs and measures, it effects a temporary lowering of blood pressure without regard to the cause of the hypertension, a result of doubtful value to the patient (44, pp. 124,128).

A widely used medical text published the same year warned of too frequent measurement of the hypertensive patient's blood pressure to prevent "hypertension hypochondriasis" as "...patients suffer more from knowing that hypertension is present than from the hypertension itself" (11, p. 1128).

Notwithstanding this therapeutic nihilism, both insurance statistics and the Framingham study independently documented a 30% increase in cardiovascular mortality and morbidity associated with each 10 mm Hg increase in blood pressure (23,62). The latter study also reported that hypertensives had 5 to 30 times the incidence of stroke compared to the normotensive control groups, four times the incidence of congestive heart failure, three times the incidence of coronary disease, and twice the incidence of occlusive peripheral arterial disease (23,61). Other studies also documented the high incidence of renal failure in patients with severe or malignant hypertension (59).

While the pathophysiology of this disorder has not been completely elucidated, the emphasis of past research was almost exclusively directed at definition of the renal-ischemic and renal-humoral mechanisms of hypertension (44). The pioneering works of Goldblatt and
others dominated the field of hypertensive investigation, but current research efforts now include investigating alterations of the central nervous system as a possible etiologic site for the development of this disorder. Indeed, the role of the central nervous system in hypertension was addressed in several recent monographs which focused particularly on the abnormal function of the sympathetic autonomic nervous system associated with hypertension (21,78). Although several studies of hypertensive patients have documented evidence of increased sympathetic nervous system activity (24,78), and while a majority of effective antihypertensive agents interfere with the function of the sympathetic nervous system (82), it remains uncertain whether the abnormal sympathetic tone is a secondary factor caused by the hypertension or whether it is a manifestation of a primary disorder of the nervous system which ultimately produces hypertension.

The traditional concept of central cardiovascular control was a hierarchical construct in which the medullary vasopressor and vasodepressor centers controlled end organs via dedicated autonomic outflow pathways. Recent research has forced modification of this concept and the incorporation of multiple descending functional pathways that mediate patterns of cardiovascular adjustments to specific demands. Although the specificity of the action of the parasympathetic system has been long recognized, the same characteristic of the sympathetic outflow was not appreciated until recently (12). The anatomical substrates of these functional pathways are the subject of continuing investigation which has established the existence of multi-
pie bulbospinal monoaminergic pathways which project upon the sym-
pathetic preganglionic neurons (SPGNs) of the intermediolateral cell
column of the spinal cord. Serotonin (5-HT), norepinephrine (NE), and
epinephrine (EPI) neuronal projections primarily from the brainstem to
the obligate output of the sympathetic system, namely the SPGNs, re-
represent mechanisms by which sympathetic outflow can be modulated (75).

The respective functional roles of the three monoaminergic path-
ways to SPGNs have not been resolved. The consensus of evidence con-
cerning the 5-HT pathways indicates that they are inhibitory to SPGNs
(9,70), but evidence of the opposite nature (20,47) raises uncer-
tainty. Iontophoretic studies have shown that NE (20,46), EPI and
clonidine (46) are inhibitory to SPGNs which is consistent with the
sympathoinhibitory effects of systemically administered L-dopa (13,48,
81,92) and clonidine (3,6,32,36,92) at the spinal level. However,
some experimental conditions reveal an excitatory effect of L-dopa
(32,35,48,81,98), and studies on experimental (10,39,78) and clinical
hypertension (21,78) suggest that the NE pathways are excitatory.
Proposals that EPI pathways are either inhibitory (40) or excitatory
(17) to SPGNs have not been resolved, but iontophoretically applied
EPI is strongly inhibitory (46). Previous studies in our laboratory
on the effects of monoamine precursors and other monoaminergic drugs
on spinal sympathetic pathways suggest that the NE and 5-HT pathways
to SPGNs are, respectively excitatory and inhibitory (32,35,81).

The present studies used cat preparations of four central sympa-
thetic pathways (see Figure 1) to elucidate the sites and mechanisms
of action of three antihypertensive agents which appear to alter the function of the central nervous system either as the major action or as a contributing factor responsible for the antihypertensive effect of the agent. Both methyldopa and clonidine were accidentally discovered to have hypotensive activities which proved to be clinically significant (7,65). Experimental studies have documented that clonidine and methyldopa reduce the sympathetic outflow but the exact site and mechanism of action have not been determined. The action of propranolol on the central nervous system is less certain although reports of such have been published (18,63,74).
MATERIALS AND METHODS

Adult cats, 2.5-4.5 kg, were made spinal at C1 under brief ether or methohexital anesthesia or were anesthetized with alpha-chloralose (60 mg/kg, i.v.). The spinal animals were rendered functionally de-cerebrate by bilateral ligation of the internal carotids and clamping of the vertebral arteries. All animals were intubated and artificially ventilated with a positive pressure respirator. End-tidal CO$_2$ concentration was continuously monitored and blood gases were periodically tested to ensure optimal ventilation. Blood pressure was monitored by an internal carotid or femoral artery cannula and maintained with periodic infusions of normal saline and dextran-40. Internal body temperature was continuously monitored with a rectal probe and maintained at 36-38 °C with a regulated heating plate. Animals were paralyzed with gallamine triethiodide (Flaxedil; 10 mg/kg plus supplements).

Preparations were held in position with a mandibular clamp and clamps on the thoracic vertebral spinous processes. The upper thoracic sympathetic ganglion chain was exposed retropleurally by removing the overlying spinal musculature. The second through fourth ribs were sectioned at their junction with transverse processes and 2-cm sections were removed. The white rami and the intercostal spinal nerves of T2-T4 were identified, dissected free and sectioned dis-

tally. A pool of mineral oil was formed over the surgical site with the intact pleura forming the bottom of the pool. Subsequent surgical preparation depended upon the pathway or pathways tested (Fig. 1). Somatospinal reflex pathways were evoked by stimulation of an exposed intercostal nerve with bipolar, silver wire electrodes (0.2 Hz, 1.5-3 V, 0.2 msec). Viscerospinal sympathetic reflex pathways required the exposure of the sympathetic chain at T8-T10 which was sectioned distally, and the proximal end was stimulated through bipolar silver wire electrodes in the extended mineral oil pool (stimulus parameters as above). The intraspinal pathway was stimulated through bipolar tungsten microelectrodes (0.1 Hz, 0.50-0.15 mA, 0.2 msec, biphasic) stereotactically positioned in the dorsolateral funiculus at C2-C3 which was exposed by laminectomy and reflection of the overlying dura and dorsal roots. Transmission through spinal-bulbospinal pathways was evoked by stimulation (0.1 Hz, 1.5-3 V, 0.2 msec) of the proximal portion of a thoracic intercostal spinal nerve in intact, alpha-chloralose anesthetized animals which were debuffered by bilateral section of the carotid sinus, aortic baroreceptor, and vagus nerves.

Evoked responses from sympathetic preganglionic neurons were recorded from the preganglionic rami with bipolar, silver wire electrodes, amplified and displayed on an oscilloscope, and analyzed on-line at 5 or 10-minute intervals by signal averaging (Nicolet 1072). Integrals of 16 or 32 consecutive responses were converted to percentages of mean control values for standardization among experiments. Experiments were conducted only after preparations stabilized during a
Figure 1. Diagrammatic presentations of the four central sympathetic pathways. Traces show typical responses evoked through each pathway and recorded from upper thoracic preganglionic rami.
SOMATOSPINAL REFLEX PATHWAY (SSR)

INTRASPINAL PATHWAY (IS)

VISCEROSPINAL REFLEX PATHWAY (VSR)

SPINAL-BULBOSPINAL REFLEX PATHWAY (SBS)
control period of at least 1 hour. In the absence of drug treatment, responses were stable for periods in excess of 6 hours. Drugs utilized in these experiments were injected through a cannula in the cephalic vein.

The following drugs were used: methohexital Na (Lilly), alphachloralose (Sigma), gallamine triethiodide (Davis-Geck), clonidine HCl (Boehringer-Ingelheim), tolazoline HCl (Ciba), yohimbine HCl (Aldrich), 5-hydroxytryptophan (ICN Nutritional Biochemicals Pharmaceuticals), aminophylline (Abbott), isobutylmethylxanthine (Aldrich), propranolol (Ayerst), methyldopa (Merck, Sharp, and Dohme), reserpine (Ciba), SKF 64139 (Smith, Kline, and French).
RESULTS

Clonidine

Depression of four central sympathetic pathways

Sympathetic discharges evoked by activation of each pathway appeared as compact multunit responses with well-defined latencies and durations. In agreement with previous studies (26,31), latencies and durations of discharges evoked by visceral afferents were several milliseconds longer than those evoked by somatic afferents (10-18 msec vs. 5-12 msec). Latencies of intraspinally evoked discharges (9-11 msec) were consistent with intraspinal conduction velocities of 4-5 m/s (9). Reflex and intraspinally evoked discharges ranged between 8 and 25 msec in duration. The latencies of spinal-bulbospinal reflexes were much longer (25-35 msec), reflecting the long afferent-efferent pathways to and from the brainstem, and the duration of discharges (15-35 msec) was longer than those evoked by the other pathways. In 6 of 15 experiments, a small, short-latency spinal reflex preceded the long-latency spinal-bulbospinal reflex.

Clonidine rapidly produced a stepwise, dose-dependent depression of transmission through each of the four pathways (Fig. 2). The amount of depression produced by a particular dose level was very consistent for each pathway and generally tended to stabilize at the
Figure 2. Average depression of somatospinal reflex (A), viscerospinal (B), intraspinal (C), and spinal-bulbospinal (D) pathways by cumulative doses of clonidine and reversal by tolazoline. Vertical lines represent S.E.M. and N is the total number of experiments.
A  
SSR  
N=3  
150 1 CLONIDINE 10 15 25 50 μg/kg  
I J f J  
100 --- ------ ------  
50  
II 1  
••  
TOLAZOLINE 2mg/kg  
IS  
N=3  
CLONIDINE 5 5 2.5 5.0 μg/kg  
~  
1  
..  
• ie·  
t  
TOLAZOLINE mg/kg  
1.0 1.5  
1.5  
0  
C  
0  
TIME-HOURS  
B  
VSR  
N=6  
150 1 CLONIDINE 10 15 25 50 μg/kg  
I J f J  
100 --- ------ ------  
50  
II 1  
••  
TOLAZOLINE 2.5mg/kg  
SBS  
N=4  
CLONIDINE 2.5 2.5 5.0 μg/kg  
0 sas  
N=4  
CLONIDINE 10 15  
10 1.5  
1.5  
0  
C  
0  
TIME-HOURS
lowest level within 5-10 minutes. However, depression of transmission through the spinal-bulbospinal pathway was somewhat more variable, and responses appeared to recover partially between successive dosage increments. Tolazolone (2-4 mg/kg) or yohimbine (0.5-1.0 mg/kg) rapidly and completely antagonized the effects of clonidine on each pathway (Fig. 2).

The somatospinal (Fig. 2A) and viscerospinal (Fig. 2B) reflex pathways were successively depressed by cumulative doses of 10, 25, and 50 ug/kg of clonidine, but could not be depressed below 40% of control values, even by additional doses. Since the depressant effects on each pathway appeared to be very similar, clonidine was also tested on both pathways in the same preparations in which the two inputs were alternated every 5 minutes (Fig. 3). In this series of experiments, both pathways were depressed equally by each dose level of clonidine, and neither pathway could be depressed below 40%, even by doses up to 200 ug/kg.

Clonidine was considerably more effective in depressing transmission through Intraspinal (Fig. 2C) and spinal-bulbospinal (Fig. 2D) pathways, and each pathway could be depressed almost completely by cumulative doses of 25 ug/kg or 10 ug/kg, respectively. In those experiments in which a short-latency spinal reflex preceeded the spinal-bulbospinal reflex, the latter reflex was clearly much more sensitive to depression by clonidine.

The quantitative differences in sensitivity to clonidine among the spinal-bulbospinal, Intraspinal, and somatospinal reflex pathways were
Figure 3. Average depression by clonidine of the viscero- and somato-spinal reflex pathways alternately stimulated in the same animal (A) and dose-response relationships for the same data (B). Vertical bars represent S.E.M. and N is the total number of experiments.
A

Average response - % control

Time (hours)

10 15 25 50 100 µg/kg

△ Somatospinal reflex

△ Viscerospinal reflex

N = 6

Tolazoline 5 mg/kg

B

Average response - percent control

Dose of clonidine (µg/kg)

10 25 50 100 200

△ Somatospinal reflex

△ Viscerospinal reflex

N = 6
evident in composite dose-response relationships derived from all experiments confined to one of the three pathways (Fig. 4). The three dose-response curves were essentially parallel over their ranges of linearity. The $ED_{50}$s calculated from these data were 2 ug/kg, 6 ug/kg, 30 ug/kg, respectively. As noted in the combined somatospinal-viscerospinal reflex studies (Fig. 3), the somatospinal reflexes were depressed to 40% of control values by 50 ug/kg of clonidine and could not be depressed further by cumulative doses up to 200 ug/kg (Fig. 4).

In order to assess a possible contribution of the anesthetic to the differences in sensitivity between the spinal-bulbospinal and the other pathways, the effect of clonidine on transmission through the intraspinal pathway was also tested in chloralose-anesthetized, spinal cats ($n=4$). Depression of intraspinal transmission by graded doses of clonidine in these experiments was nearly identical to that obtained in unreanesthetized, spinal animals (Fig. 4).

**Comparison with 5-hydroxytryptophan**

Previous studies have shown that serotonin (5-HT) precursors gradually depress transmission through both spinal reflex and intraspinal pathways and that these effects are potentiated by inhibition of monoamine oxidase or 5-HT reuptake but are prevented by inhibition of central decarboxylase (35,36,42,81,90). These findings indicate that the depression is mediated by 5-HT spillover, most likely from the 5-HT terminals adjacent to sympathetic preganglionic neurons.
Figure 4. Dose response relationships for depression of three central sympathetic pathways by clonidine (S.E.M. bars included). Number of measurements at each dose level in brackets.
SPINAL-BULBOSPINAL REFLEX
• INTRASPINAL PATHWAY
• SHORT SPINAL REFLEX

AVERAGE RESPONSE – PERCENT CONTROL

DOSE OF CLONIDINE (µg/kg)
These studies demonstrated marked similarities between the depressant effects of clonidine and of the 5-HT precursors, L-tryptophan and 5-hydroxytryptophan (5-HP), suggesting that clonidine might act at the spinal level by activating postsynaptic 5-HT receptors on SPGNS (36). However, mounting evidence that clonidine binds selectively to central alpha2-adrenergic receptors (103,105) and is not displaced by 5-HT (79) prompted this part of the present study designed to examine more definitively the similarity of their effects on spinal sympathetic pathways.

Since 5-HP is considerably more effective than L-tryptophan, several dose levels of 5-HP were tested on each pathway. As shown in Figure 5, 30 mg/kg of 5-HP produced a modest depression of transmission through both pathways that recovered by 3 hours. Doses of 50 mg/kg produced a greater depression with no recovery by 3 hours. In contrast to the relative potency of clonidine on the two pathways, 5-HP was somewhat more effective on the spinal reflex than on the intraspinal pathway. In addition, transmission through both pathways could be depressed almost completely, the spinal reflex by 50 mg/kg and the intraspinal pathway by 100 mg/kg (Fig. 6). Although tolazoline or yohimbine, in doses 5 to 10 times larger than those required to antagonize equivalent depression by clonidine, appeared to reverse the depressant effects of 5-HP in some experiments, reversal was slow and was frequently incomplete or absent. Furthermore, the antagonists alone were found to enhance transmission through both pathways in some experiments.
Figure 5. Depression of transmission through spinal reflex (A) and intraspinal (B) pathways by 30 and 50 mg/kg 5-HTP. Vertical bars represent S.E.M. for points at 0.5 hr intervals. N is the number of experiments.
Figure 6. Dose-response relationships for the depressant effects of clonidine (A) and 5-HTP (B) on intraspinal and spinal reflex pathways. Vertical bars represent S.E.M. and numbers are the total experiments at each dose.
The marked differences between the depressant effects of clonidine and of 5-HTP on transmission through spinal reflex and intraspinal pathways are evident from the composite dose-response relationships derived from all experiments on both pathways in Figure 6. The ED$_{50}$s of 5-HTP were 32 mg/kg for the spinal reflex pathway and 44 mg/kg for the intraspinal pathway whereas those of clonidine were 30 ug/kg and 6 ug/kg, respectively. In addition, the respective dose-response relationships were far from parallel.

A further indication that clonidine and 5-HTP depress sympathetic transmission by different mechanisms is shown in Figure 7. Following the maximal level of depression of spinal reflexes to 40% of control values by a total dose of 100 ug/kg of clonidine, administration of consecutive doses of 5-HTP produced complete depression.

**Alteration of the effects of methylxanthines**

Aminophylline (10-50 mg/kg) or isobutylmethylxanthine (IBMX; 0.5-2.0 mg/kg) rapidly and markedly enhance transmission through intraspinal pathways within 5 minutes (84). As shown in Figure 8A and B, the intermediate doses of each drug (25 mg/kg and 1.0 mg/kg) produced maximum enhancement within 20-30 minutes, after which the size of the evoked responses declined to control levels by about 1.5 hours.

Clonidine (25 ug/kg) not only produced a typical depression of intraspinal transmission but also completely prevented enhancement by aminophylline (Fig. 8C) or IBMX (Fig. 8D). A similar effect was dem-
Figure 7. Complete depression of spinal sympathetic reflexes by 5-HTP after maximum depression by clonidine. Vertical bars represent S.E.M. The 5-HTP was infused for 15 min in these four experiments.
SPINAL REFLEX

PERCENT CONTROL

TIME - HOURS

CONTROL

5-HTP
30mg/kg

5-HTP
20mg/kg

25 75µg/kg
CLONIDINE

N=4
Figure 8. Average effects of aminophylline and IBMX alone (A and B), after 25 μg/kg of clonidine (C and D), and after toladoline (E) or yohimbine (F) on intraspinal transmission to sympathetic preganglionic neurons. Upper traces in A are single responses and lower traces are averages of 16 responses, before and 30 min after 25 mg/kg of aminophylline, from one of the 3 experiments shown in the graph; calibrations, 25 msec and 50 μV. The intraspinal pathway is depicted in E.
Aminophylline 25 mg/kg
N=3

T3

IBMX 1.0 mg/kg
N=4

Clonidine 0.025 2.5
Tolazoline

Amin.

25

Clonidine Tolazoline

0.025 2.5

IBMX 1.0

Yohimbine 1.0

N=4

N=2

N=4
onstrated on the somatospinal reflex pathway (Fig. 9). The alpha₂-receptor antagonists, tolazoline or yohimbine, typically produce prompt reversal of clonidine-induced depression back to control levels (36). As shown in Figures 8C and D, tolazoline or yohimbine not only antagonized the depression of clonidine but restored the ability of the xanthines to enhance transmission and prevented the expected early decline in enhancement. Following pretreatment by tolazoline or yohimbine, the enhancement by the xanthines was not terminated in the expected 1.5 hours but continued to increase for more than 5 hours (Fig. 8E and F).

Methyldopa

Depression of central sympathetic pathways

Methyldopa was tested on three sympathetic pathways: spinal-bulbo-spinal, intraspinal, and somatospinal reflex. The drug was given over approximately 20 minutes by i.v. infusion. A modest increase was seen in the response in each pathway and no depression was seen over a time period in excess of 5 hours. However after methyldopa pretreatment, reserpine given in a dose (5 mg/kg) known to release transmitter from monoaminergic nerve terminals (15) rapidly and promptly depressed transmission through each pathway (Fig. 10). This depression was not due to reserpine since this dose given alone produces only a modest enhancement of transmission. Reserpine was ineffective in producing depression if less than 45 minutes had elapsed between methyldopa
Figure 9. The enhancement of transmission through the spinal reflex pathway by aminophylline and the complete blockade by clonidine. Vertical bars are S.E.M. and numbers equally the total numbers of experiments.
SPINAL REFLEX

CLONIDINE 100μg/kg

AMINOPHYLLINE 50 mg/kg

N=6

N=3

TIME - HOURS

AMINOPHYLLINE 50mg/kg
Figure 10. The effects of methyldopa before and after reserpine injection on the following central sympathetic pathways: spinal reflex (SR), intraspinal (IS), and spinal-bulbospinal (SBS). Recovery of response after yohimbine. Vertical bars represent S.E.M. and N equals the total number of experiments at each dose.
METHYLDOPA RESERPINE
150 5.0 mg/kg

YOHIMBINE 0.5 mg/kg

TIME (HOURS)
pretreatment and the injection of reserpine. These results are consistent with previous observations that methyldopa exerts its sympathoinhibitory effects via a metabolite (65).

Although an insufficient range of doses of methyldopa were tested to produce a meaningful dose-response relationship, it is of interest that the intraspinal and spinal-bulbospinal pathways were completely inhibited by the standard 150 mg/kg dose tested but that the somatospinal reflex pathway was incompletely depressed (Fig. 10). Indeed, the spinal reflex pathway was depressed to only 40% of control by a larger dose of methyldopa (200 mg/kg). In two spinal-bulbospinal pathway experiments in which short latency spinal reflexes were also present, the differential sensitivity of these two pathways to methyldopa was clearly demonstrated (Fig. 11).

**Phenylethanolamine-N-methyltransferase Inhibitors**

Pretreatment of animals with the phenylethanolamine-N-methyltransferase (PNMT) inhibitor, SKF 64139, which produces marked depletion of central epinephrine (38), significantly altered the effects of methyldopa on transmission through both intraspinal and somatospinal reflex pathways. Treatment with SKF 64139 typically produces an immediate but transient depression of transmission through each pathway which appears to be unrelated to PNMT inhibition (Fig 12; Sangdee and Franz, unpublished observations).
Figure 11. The effect of methyldopa on both spinal-bulbospinal (SBS) and a simultaneous spinal reflex (SR) transmission of the same intact animal. The incomplete depression of transmission in the spinal reflex pathway after treatment with reserpine and the reversal of the depression by yohimbine.
METHYLDOPA RESERPINE

150 mg/kg  5.0 mg/kg

RESPONSE (% CONTROL)

TIME (HOURS)

N = 2

SBS
SR

YOHIMB. 0.5
Figure 12. The reversal of methyldopa-induced depression of intraspinal (IS) and spinal reflex (SR) pathways by pretreatment with PNMT inhibitor. Clonidine and yohimbine demonstrate that alpha,,-receptors are not blocked. Vertical line represent the S.E.M. and N is the number of experiments.
After pretreatment with SKF 64139, methyldopa infusion produced a strikingly different response on the intraspinal pathway in which transmission was gradually but markedly increased to approximately 600% of control values. In addition, the subsequent reserpine treatment did not produce the typical depression of either pathway and the spinal reflex responses were actually enhanced (Fig. 12). The enhancements by methyldopa were not due to alpha₂-receptor blockade by SKF 64139 (99), because clonidine was still able to depress transmission through both pathways. In these experiments, the enhancement by methyldopa could be blocked by chlorpromazine.

Propranolol

Propranolol was also tested on three central sympathetic pathways: spinal-bulbospinal, intraspinal, and somatospinal. In each pathway transmission was gradually but definitely enhanced by propranolol (Fig. 13). The enhancement was quite variable and did not appear to be dose-related.
Figure 13. The enhancement of three central sympathetic pathways by propranolol: spinal reflex (SR), intraspinal (IS), and spinal-bulbo-spinal (SBS). Vertical bars represent S.E.M. and N is the number of experiments.
SR N=4
PROPRANOLOL
5 mg/kg

IS N=7
PROPRANOLOL
2.5 mg/kg

SBS N=5
PROPRANOLOL
5 mg/kg

RESPONSE - % CONTROL

TIME - HOURS
DISCUSSION

The present results demonstrate that transmission through the descending pathways to sympathetic preganglionic neurons is considerably more vulnerable to the depressant effects of clonidine than is that through spinal reflex pathways which normally serve a minor role in regulating the sympathetic outflow. Not only are both intraspinal and spinal-bulbospinal pathways depressed by much lower doses than required for the spinal reflex pathways, but the descending pathways can be depressed completely whereas spinal reflexes are maximally depressed to only 60% of control values. The rapid reversal of this depression by alpha$_2$-receptor antagonists is consistent with previous studies and with radioligand binding studies which show a highly selective affinity of clonidine for central alpha$_2$-receptors (79,103) which appear to be postsynaptic (101). Since both descending and spinal reflex pathways are depressed in parallel, it appears that the inhibitory alpha$_2$-receptors are located on sympathetic preganglionic neurons (SPGNs).

Conventional inspection of the dose-response curves (Fig. 4) suggests that the spinal-bulbospinal pathway is about three times more sensitive to clonidine than the intraspinal pathway (e.g., $E_{50}$ 2 vs. 6 ug/kg). However, since the intraspinal pathway includes the efferent limb of the spinal-bulbospinal pathway, quantitative differential-
tion of the supraspinal sites by this standard approach may not be totally adequate.

The depressant effect of clonidine on the intraspinal pathway is exerted only at the spinal level whereas it is exerted simultaneously at both spinal and supraspinal levels on the spinal-bulbospinal pathway. Therefore, a different type of comparison can be made by considering the total effect of a particular dose of clonidine on the spinal-bulbospinal pathway in terms of the contribution made by its effect only on the efferent intraspinal pathway. For example, 5 ug/kg depressed transmission through the intraspinal pathway by 45% and through the spinal-bulbospinal pathway by 72%, only an additional 27%. The 10 ug/kg dose produced 65 and 88% depression, respectively, for a similar increment of only 23%. Therefore, these data suggest that more of the total depressant effect of clonidine on the spinal-bulbospinal pathway is exerted at the spinal level than at the brainstem level. A similar analysis of data obtained from splanchnic nerve potentials evoked through these two pathways (3) also suggests that clonidine has a greater effect at spinal than at supraspinal sites. However, extrapolation of the data indicates that spinal-bulbospinal pathways would be depressed at lower dose levels than those affecting intraspinal transmission. Therefore, neither type of analysis appears to be completely adequate for quantitative differentiation of relative depressant actions on different sites in the same pathway. Nevertheless, the data suggest that the spinal sites are about as sensitive to clonidine as the relevant supraspinal sites and that both make a significant contribution to depression of the spinal-bulbospinal reflex.
The greater depression of spinal-bulbospinal reflexes cannot be attributed to additive effects with the anesthetic because the intraspinal pathway was equally depressed by clonidine in both anesthetized and unanesthetized spinal cats. However, it should be recognized that intraspinal stimulation may provide a more powerful activation of preganglionic neurons than does the longer, more complex spinal-bulbospinal pathway. The longer afferent, connecting, and efferent pathways all contribute to a greater asynchrony and temporal dispersion of the final input to spinal centers; this factor probably accounts for the longer duration of discharges evoked through the spinal-bulbospinal pathway. Therefore, the spinal effect of clonidine might actually be greater on the longer pathway than on the shorter, more direct intraspinal pathway. This difference would, in effect, tend to amplify rather than diminish the sensitivity of the spinal sites to clonidine as determined in the present experiments. However, considerable evidence indicates that clonidine exerts prominent cardiovascular effects at supraspinal sites (58,66), and the present findings do not preclude a somewhat different proportion of effects at the two sites when all systems are operative. Other sympathetic reflexes mediated through the brainstem (e.g., chemoreflexes) may differ in their respective sensitivities to depression by clonidine. Nevertheless, since all central sympathetic activity must be routed through the spinal pathways to SPGNs, further prominent depression by clonidine at this site is imminent regardless of the supraspinal origin.
The much lower sensitivity and limited effect of clonidine on both spinal reflex pathways suggests that the relationship of the alpha₂-receptors to the incoming reflex pathways differs from that to the descending pathways. This may reflect differences in their transmitters or neuromodulators, in the location of their terminals (e.g., somatic vs. dendritic), or in their synaptic coupling or circuitry. Whatever the reason, these differences suggest that spinal reflex and descending pathways are separate and do not share common elements. The observation that clonidine produces identical effects on somato- and viscerospinal sympathetic reflexes is consistent with previous evidence that both spinal inputs traverse common interneurons in their respective pathways (26,85,91).

The results of this study also agree with those of other investigators (67,69,92) including several who have also noted the lesser sensitivity of spinal reflex than of descending pathways to depression by clonidine (3,6). Spinal reflexes for electrodermal responses (6) and for evoked splanchnic nerve discharges (3) are considerably less sensitive than are the corresponding spinal-bulbospinal reflexes. However, in comparison to the present results, both electrodermal reflexes are much more sensitive and can be depressed completely, and their relative sensitivities differ by less (ED₅₀ s, 2.0 and 0.6 µg/kg). On the other hand, reflexes recorded from splanchnic nerves are less sensitive (ED₅₀ s, 100 and 16 µg/kg) than found in the present study, but the depression of both spinal reflexes and intraspinally evoked discharges appears to be limited. In a study on caudal spinal
sympathetic reflexes (69), reflexes between splanchnic and hypogastric or renal nerves or between pelvic and hypogastric nerves were almost completely depressed by 50-60 µg/kg of clonidine, but reflexes to lumbar colonic nerves were not affected even by much larger doses.

These differing sensitivities of evoked sympathetic activity to depression by clonidine appear to depend on the type or spinal level of sympathetic outflow measured. These differences suggest that the various functional pools of sympathetic neurons differ markedly in their responsiveness to clonidine, probably reflecting differences in their number or distribution of alpha₂-receptors. Such differences may contribute to selective regulation of the diverse autonomic functions by the central nervous system.

The results of this study confirm previous reports that 5-HTP markedly depresses transmission through both the intraspinal and somatospinal sympathetic reflex pathways. This more complete analysis of the effect of 5-HTP indicates that clonidine does not depress these pathways by activation of serotonin (5-HT) receptors. The relative sensitivities of the intraspinal and somatospinal pathways to clonidine and 5-HTP are completely opposite to one another. Unlike clonidine, 5-HTP is somewhat more effective in depressing the spinal reflex pathway than the intraspinal pathway and both pathways can be completely depressed. After maximal depression of the spinal reflex by clonidine, 5-HTP is able to depress this pathway completely. The dose-response curves for depression of both pathways by each drug are essentially parallel, but those between drugs are far from parallel.
Unlike clonidine, the depression of the two central pathways by 5-HTP is slowly and incompletely reversed by tolazoline and yohimbine. This suggests that the reversal is the result of nonselective secondary effects on other factors not directly related to the blockade of alpha₂-receptors. The contrasting results indicate that the depression of the sympathetic pathways by clonidine and by 5-HTP are due to the activation of different receptors: alpha₂ and 5-HT, respectively.

The present study demonstrates that depression of sympathetic pathways by 5-HTP is linearly related to dose and can be virtually complete in the spinal cat. A number of investigators have reported that administration of 5-HTP to various species reduces blood pressure (2,28,53) and sympathetic activity (3,97). Similar effects of L-tryptophan have been observed only when given with a monoamine oxidase inhibitor (28). The present study demonstrates that the spinal level is an important site of the vasodepressor and sympathoinhibitory effects of 5-HT precursors and that this level is probably important in the mediation of sympathetic integration in intact animals. Since the sympathetic preganglionic neurons are common to both spinal and intraspinal pathways, these neurons provide the most likely sites for the depressant action of 5-HT released from adjacent 5-HT terminals.

The enhancement of intraspinal transmission by aminophylline and IBMX appears to be due to inhibition of phosphodiesterase rather than to some other action of xanthines. Their difference in potency (about 50 fold) is consistent with their relative potencies as phosphodiesterase inhibitors in vitro (16) but not with their similar affinities
as antagonists for adenosine receptors (16,95). A nonselective effect on neuronal excitability also seems unlikely since even the maximum doses tested did not produce spontaneous discharges in either preganglionic rami or lumbar ventral roots and had no effect on spinal somatic mono- or polysynaptic reflexes (33). Instead, the xanthines appear to enhance intraspinal transmission by preserving cyclic adenosine-monophosphate (cAMP) which increases the excitability of SPGNs, possibly by phosphorylation of membrane proteins (45,88). Since phenothiazines effectively block central NE receptors (37), the ability of chlorpromazine to impair markedly both intraspinal transmission and its enhancement by phosphodiesterase inhibitors suggests that cAMP may be generated by the NE pathway through adrenergic receptors that are positively coupled to adenylate cyclase. These receptors do not appear to be beta-adrenergic receptors because propranolol modestly enhances rather than depresses the intraspinal transmission (76).

Radioligand binding studies and the selective antagonism of the central effects of clonidine by alpha2-receptor blocking drugs indicate that clonidine acts on alpha2-receptors which are largely post-synaptic in the central nervous system (102) and are negatively coupled to adenylate cyclase in human platelets, in neuroblastoma glioma hybrid cells (1,89), and rat cerebral cortex slices (93). The present results suggest that depression of preganglionic neurons by clonidine is also mediated by activation of alpha2-receptors that are negatively coupled to adenylate cyclase, thereby blocking synthesis of cAMP by the NE pathways and reducing intracellular levels of cAMP.
The finding that alpha\textsubscript{2}-receptor antagonists not only block the effects of clonidine but also prevent the early termination of enhancement by the xanthines suggests that a negative feedback system operates through the alpha\textsubscript{2}-receptors to limit the increased neuronal excitability produced by cAMP. Blockade of these SPGN receptors appears to inactivate the feedback system so that cAMP synthesis, the increased excitability, or both proceed unimpeded (Fig. 8E and F).

These alpha\textsubscript{2}-receptors may also function as terminal receptors for a bulbospinal inhibitory system since it has been shown that yohimbine (0.5 mg/kg) markedly potentiates electrodermal and nictitating membrane reflexes and increases heart rate in intact and decerebrate but not in spinal cats (68). Simultaneous activation of these inhibitory pathways in several of the present experiments may account for the increased transmission by tolazoline alone (Fig. 8E).

The evidence for descending inhibitory and intraspinal negative feedback systems that operate through spinal alpha\textsubscript{2}-receptors prompts consideration about the nature of an endogenous ligand and its normal functional role. Several factors suggest that epinephrine might fulfill this role as an inhibitory transmitter or modulator. Bulbospinal epinephrine (EPI) pathways appear to terminate almost exclusively in the intermediolateral columns (56) suggesting that they have a selective function in this region. This arrangement takes on added significance by the findings that neither clonidine (36) nor the xanthines (33) affect transmission through spinal somatic pathways in doses considerably larger than those which markedly alter transmission through
spinal sympathetic pathways (34). In addition, the affinity of EPI for brain alpha$_2$-receptors is 3-4 times that of NE (102). Independent activation of negatively coupled alpha$_2$-receptors by EPI and of positively coupled adrenergic receptors by NE would allow the two catecholamine pathways to modulate the excitability of SPGNs by either suppressing or activating adenylate cyclase.

The independent activation of different adrenergic receptors on the same neurons by two closely related catecholamines would require a high degree of synaptic and receptor isolation. Such an arrangement in SPGNs is rendered plausible by the primarily axodendritic location of NE terminals (42,94) and the probable somatic location of alpha$_2$-receptors responding to iontophoretic application of EPI, NE, and clonidine (20,46). Although NE can activate the inhibitory alpha$_2$-receptors on the small soma, the terminal distribution of NE pathways to the extensive dendritic branches (22,87) would normally limit NE release to the excitatory adrenergic receptors. However, since the dendrites innervated by NE terminals are largely confined to the SPGN neuropil (14,22,87), the normally excitatory NE pathways might become self-regulating during excessive release by activating the alpha$_2$-receptors to reduce the level of SPGN excitability. In their strategic location on or near the cell soma, the alpha$_2$-receptors appear to dominate neuronal excitability regardless of input to other receptors.

The ability of alpha$_2$-receptor antagonists to prevent the early termination of enhancement by the xanthines (Fig. 8E and F) represents another type of self-regulation. These results suggest that the nega-
tive feedback system may involve depolarization of EPI terminals by collateral pathways which release EPI to activate alpha$_2$-receptors thereby limiting the increased excitability produced by high levels of cAMP.

The proposal that EPI and NE pathways control the excitability of SPGNs by exerting opposite effects on adenylate cyclase from different parts of the neuron may appear somewhat inconsistent with classical views of receptor-enzyme coupling. However, recent evidence that membrane receptors in many cells are coupled to adenylate cyclase through different guanosine-triphosphate (GTP)-regulatory proteins which either stimulate or inhibit its activity (88) provides a mechanism for reciprocal regulation by spatially separated receptors. Whatever the mechanism, the present results furnish evidence for such a regulatory process in SPGNs whereby the intraneuronal levels of cAMP are controlled by activation or inhibition of adenylate cyclase. It seems highly probable that the excitability of certain other central neurons may be similarly regulated. (See Figure 14).

Although clonidine is clearly less effective on both spinal reflex pathways than on descending pathways, the present results strongly suggest that it may be effective in controlling the symptoms of autonomic dysreflexia which commonly afflicts patients with high spinal cord lesions (60,64). These symptoms are produced by hyperactive spinal sympathetic reflexes evoked by cutaneous or visceral stimulation. Clonidine does not affect the blood pressure of tetraplegic patients at rest (86), presumably because spinal sympathetic tone is ab-
sent, but it markedly reduces the acute hypertensive episodes evoked by bladder distension in such patients (77). The present results predict that clonidine would depress hyperactive reflexes evoked by either visceral or cutaneous stimulation about equally, but would not depress them so completely that their value as an important alarm sign of visceral disturbances (30) would be abolished. The incomplete inhibition of sympathetic reflex activity by clonidine is also desirable because less postural hypotension is produced by clonidine than other currently employed agents which can produce more complete reduction in sympathetic outflow at therapeutic doses (8,30).

The hypotensive effect of methyldopa is the result of the inhibition of sympathetic outflow at an undefined central site (19,27,54,55,57). This action is prevented by inhibition of central but not of peripheral aromatic aminoacid decarboxylase (51,52) and by inhibition of dopamine-beta-hydroxylase (19,50). Alpha-methylnorepinephrine (MNE) is considered to be the active metabolite of methyldopa and is thought to interact with central alpha_2_-receptors since alpha_2_-receptor antagonists prevent or reverse the hypotensive action of methyldopa (49). Although MNE has been shown to bind alpha_2_-receptors and is considered to be a "false" transmitter, a recent study of the effects of systemic and intrathecal administration of MNE demonstrated a pressor response equipotent to systemic i.v. NE and a prolonged pressor response after intrathecal administration in the unanesthetized rhesus monkey (29). These experiments and others do not support the theory that MNE is the active inhibitory metabolite of
methyldopa (100). The current study was undertaken to define further the site and mechanism of action of methyldopa in light of the results of the above described studies with clonidine and the hypothesis regarding the opposing actions of descending bulbospinal EPI and NE pathways.

A slow i.v. infusion of methyldopa produced moderate enhancement of transmission through each of the sympathetic pathways tested, which persisted for periods in excess of 5 hrs. Since other investigators had reported that methyldopa depressed lumbar sympathetic reflex pathways in intact anesthetized cats (4), reserpine was given in a dose sufficient to release monoaminergic transmitters. When given after the methyldopa infusion, reserpine caused a prompt depression of transmission in all three pathways, and this depression could be reversed or prevented by the alpha₂-receptor antagonists, yohimbine and tolazoline. The depression was not produced by reserpine because reserpine only modestly increases rather than decreases transmission through each pathway when given alone. The fact that reserpine was ineffective in producing depression if less than 45 minutes had elapsed since methyldopa infusion is consistent with the view that methyldopa acts via a metabolite.

These experiments demonstrate several similarities between the actions of methyldopa and clonidine. Not only are the depressant effects of each agent reversed by alpha₂-receptor antagonists, but there appears to be a differential sensitivity of the three sympathetic pathways. Although an insufficient range of dosages of methyldopa was
tested to produce dose-response data for each of the pathways, the results suggest that the spinal reflex pathways are the least sensitive to methyldopa and that the spinal-bulbospinal reflex pathways may be the most sensitive.

Since the role of MNE as the inhibitory metabolite of methyldopa is not established, and since alpha-methylepinephrine (ME) has recently been demonstrated to be formed in the brain (5) and to have a high affinity for the inhibitory alpha₂-receptors (43), the current study included experiments in which central EPI stores were depleted and PNMT activity was inhibited by SKF 64139. The rapidity of the initial depression by SKF 64139 indicates that this effect of the drug is unrelated to inhibition of PNMT. After depletion of EPI by inhibition of PNMT, the infusion of methyldopa produced a gradual return to control values in the spinal reflex pathway and a gradual but very striking increase in transmission in the intraspinal pathway. In contrast to the previous experiments (Fig. 10), reserpine then modestly increased transmission through each pathway. Both clonidine and chlorpromazine depressed the enhancement of transmission through both pathways demonstrating that the alpha₂-receptors were not blocked and that the enhancement of transmission was due to activation of the central receptors thought to be innervated by the descending NE pathways (see Fig. 14). Therefore, the results suggest that MNE produces excitatory effects at the excitatory NE receptors.

These experiments support the proposal that the inhibitory metabolite of methyldopa is ME formed in EPI terminals and that it is
either released to interact directly with the \( \alpha_2 \)-receptors on SPGNs or it causes release of EPI from the descending bulbospinal EPI pathways; perhaps both mechanisms operate. The fact that depression was not seen in these experiments until reserpine induced release of the active metabolite may be due to the low rate of activation the descending bulbospinal pathways or to the acute nature of the experiments.

The present study also offers a mechanism for the infrequent observation of delayed paroxysmal hypertension after i.v. administration of methyldopa (25, 100). This paradoxical response may reflect facilitatory effects of MNE on sympathetic outflow. A hypertensive episode could occur if the pressor action of MNE was unopposed because of defective synthesis of ME or of EPI thereby compromising the effective operation of the feedback self-regulation mechanism (See Fig. 14).

Propranolol does not appear to have any acute sympathoinhibitory action at the levels tested. The mechanism of the modest enhancement of transmission is not clear, but recent reports that propranolol binds to 5-HT receptors may offer a partial explanation for these observations (80, 104). This facilitation of sympathetic transmission through the sympathetic pathways thereby increasing the sympathetic outflow offers an explanation for the reported elevation of mean blood pressure in tetraplegic patients subjected to bladder stimulation (96).

The results of the present study can be explained by the proposed mechanism outlined in Figure 14. The SPGNs appear to be a site of
Figure 14. Schematic representation of the proposed mechanism by which descending NE and EPI pathways regulate the excitability of SPGNs through reciprocal effects on adenylate cyclase. PDE, phosphodiesterase; CPZ, chlorpromazine.
action of clonidine and of the active metabolite of methyldopa. Descending bulbospinal pathways, including NE, EPI, and 5-HT, exert excitatory or inhibitory effects on these neurons. Adenylate cyclase appears to be inhibited by descending EPI neurons via \( \alpha_2 \)-receptors and to be stimulated through receptors activated by NE neurons. The EPI neurons and the \( \alpha_2 \)-receptors also provide a functional negative feedback mechanism for controlling the excitability of the SPGN pool and it seems probable that the excitability of certain other central neurons may be similarly regulated. The present study suggests that clonidine and methyldopa exert a substantial part of their hypotensive action via stimulation of \( \alpha_2 \)-receptors on SPGNs to reduce sympathetic outflow.
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<thead>
<tr>
<th>Name</th>
<th>Parley William Madsen III</th>
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<tbody>
<tr>
<td>Birthplace</td>
<td>Vallejo, California</td>
</tr>
<tr>
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<td>5 May 1947</td>
</tr>
<tr>
<td>High School</td>
<td>Olympus Senior High School</td>
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<td></td>
<td>Salt Lake City, Utah</td>
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<td>University</td>
<td>University of Santa Clara</td>
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<tr>
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<td>Santa Clara, California</td>
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<tr>
<td></td>
<td>B.S. (Chemistry; ACS cert.)</td>
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<tr>
<td>Honors</td>
<td>Poling Clinic Scholarship</td>
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<tr>
<td></td>
<td>Friends University, Wichita, Kansas</td>
</tr>
<tr>
<td></td>
<td>Honors Assistant</td>
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<tr>
<td></td>
<td>Department of Chemistry</td>
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<tr>
<td></td>
<td>Friends University, Wichita, Kansas</td>
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<tr>
<td></td>
<td>NIH Predoctoral Trainee</td>
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<td></td>
<td>Department of Pharmacology</td>
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<td></td>
<td>University of Utah</td>
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<td>School of Medicine</td>
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<td>Medical student member of Retention and Tenure Committee.</td>
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</table>
Honors Program
Department of Neurology
University of Utah
School of Medicine

Professional Organizations

American Medical Student Association
American Medical Association
Society for Neuroscience
American Chemical Society

Professional experience

Teaching assistant
Department of Chemistry
Friends University
Wichita, Kansas
1967-1968

Teaching assistant
Department of Chemistry
University of Santa Clara
Santa Clara, California
1974-1975

Research Assistant
Department of Chemistry
University of Santa Clara
Santa Clara, California
1974-1975

Research Technician
Department of Pharmacology
University of Utah
School of Medicine
1974-1975

Research Associate
Department of Pharmacology
University of Utah
School of Medicine
1980-1982
Publications

Papers


Abstracts


