Effects on Brain Biogenic Amines of Repeated Treatments with Calcium Antagonists

Renato Gaggi and Anna Maria Gianni

Institute of Pharmacology, University of Bologna, via Irnerio 48, 40126 Bologna, Italy

Received November 26, 1990 Accepted February 26, 1991

ABSTRACT — Discrete brain sections were obtained from rats once or repeatedly (once a day for 5 days) given i.p. nifedipine, verapamil or diltiazem at doses ranging from 5 to 20 mg/kg. The biogenic amines and metabolites in the hypothalamus, brainstem, hippocampus, striatum, cortex and thalamus-midbrain were determined by high-performance liquid chromatography with electrochemical detection. The drug-induced changes, displaying regional specificity and differences according to the various compounds, suggested that: (a) serotonergic systems were activated, especially in fasted rats or after repeated treatment; (b) the dopaminergic system of the striatum was inhibited by nifedipine which did reduce the HVA levels and the HVA/DA, as well as the DOPAC/DA, ratio. These effects disappeared after repeated treatment. It was also speculated that the data obtained could be of great interest in view of the possible use of calcium antagonists to treat disorders of the central nervous system.

In a previous study (1), we observed that acute administration of calcium antagonists to rats induced several changes in the biogenic amines and metabolites content of various brain areas. The effects of flunarizine, being similar to those induced by neuroleptic drugs (2), markedly differed from the effects of the calcium antagonists selective (3, 4) for the voltage-sensitive calcium channels (VSCC). However, the effects of verapamil, diltiazem and nifedipine displayed regional specificity and differences according to the various compounds. On the other hand, each of these calcium antagonists binds to a specific recognition site in the macromolecular complex of the VSCC (5). Therefore, it could be argued that the VSCC of the various brain areas display differences which are determining factors of different affinity for the compounds. However, verapamil, diltiazem and nifedipine could have produced different effects also by indirect actions or by interactions with receptors other than VSCC (6–8) or Ca²⁺ channels of the L type (9). In our previous study (1), it was concluded that only some of the effects induced by acute administration of calcium antagonists could be ascribed with sufficient certainty to VSCC blockade. On the other hand, it had been shown that calcium antagonists interact with neuronal channels of the L type, while the N or T types of VSCC are unaffected by these compounds (10). Since it has been hypothesized (11) that L channels could play a role in the long-term regulation of neuronal functions, we studied the effects of verapamil, diltiazem of nifedipine, daily administered for a specific number of days, on the content of biogenic amines and their metabolites in discrete areas of the rat brain.

MATERIALS AND METHODS

Animals and general procedures

A total of 80 male Sprague-Dawley rats
(Nossan, Milan, Italy), weighing 180–200 g, were used. They were maintained on standard laboratory food and controlled conditions of light (7:00 a.m.–7:00 p.m.), temperature (22 ± 2°C) and humidity (60%). To avoid circadian variations in the brain content of biogenic amines, all rats were decapitated in the afternoon (4:00–6:00 p.m.). The brain was rapidly removed and ice-cooled. The cerebellum and the olfactory tubercles were discarded, whereas the hypothalamus, the hippocampus, the striatum, the brainstem (pons plus medulla oblongata) and the cortex were dissected, immediately frozen with pulverized dry ice and kept at −80°C until the time of analysis. The remaining cerebral tissue, consisting mostly of the thalamus and midbrain, was also collected, frozen and analyzed. All the samples from the same experiment were analyzed within 10 days after collection. The same brain section of each animal was analyzed on the same day, repeating complete replications sequentially.

Drugs and treatments

Nifedipine and diltiazem hydrochloride were kindly supplied by Schiapparelli Farmaceutici (Turin, Italy). Verapamil hydrochloride was from Sigma Chemical Co. (St. Louis, MO, U.S.A). Verapamil and diltiazem were dissolved with saline, while nifedipine was suspended in 1% Tween 80. The dose per kg body weight was contained in 2.0 ml. All the drugs were administered i.p. Three groups of five rats were treated with nifedipine at the dose of 0 (vehicle), 5 or 20 mg/kg for five days. Another group of five rats received nifedipine at 20 mg/kg only on the fifth day. After the last treatment, 2 hr before killing, all the rats were deprived of food. The procedure was repeated with verapamil or diltiazem, but verapamil was administered at the doses of 5 or 10 mg/kg. A fourth experiment was performed with four groups of five rats. Two groups, after 6-hr fasting, were treated with vehicle or nifedipine at 20 mg/kg. Another two groups treated in the same way were food deprived only after the treatment. All the treatments were performed 2 hr before killing.

Analytical procedure

The determination of norepinephrine (NE), dopamine (DA), 5-hydroxytryptamine (5-HT), and the metabolites, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindole 3-acetic acid (5-HIAA) was performed simultaneously by high-performance liquid chromatography with electrochemical detection (12, 13). The procedure has been previously described in detail (14).

Statistical analysis

Analysis of variance (ANOVA) was applied to all the data. This analysis was followed, in the first three experiments, by the two-tailed Dunnett's t-test in order to compare individual groups to the control. In the fourth experiment, the resolution of the “between groups” degree of freedom was performed to test the interaction “fasting/nifedipine” as well as the effect of fasting or the effect of the drug on fasted or non-fasted rats.

RESULTS

Effects of nifedipine, verapamil or diltiazem after repeated administration

The single or repeated treatments with the drugs, being well-tolerated, did not induce any change in the behavior or in the general condition of all the rats.

The data concerning the NE content in all the brain areas were not reported in the tables because they showed no significant changes. For the same reason, also the data regarding the content of DA, 5-HT and their metabolites in both hypothalamus and brainstem were not shown.

The highest dose of nifedipine (Table 1), especially by repeated administration, enhanced the 5-HIAA levels only in the hippocampus and the thalamus-midbrain. However, the 5-HIAA/5-HT ratio was also raised in the other brain areas. The HVA levels, being affected only by the single administration, decreased in the striatum alone. The repeated administration of verapamil (Table 2) increased, according to the dose, the 5-HT or
Table 1. Content of DA, 5-HT and their metabolites in brain sections obtained from rats treated with nifedipine

| Doses mg/kg | Days of treatment | Tissue levels: µg/g wet weight | DOPAC DA × 100 | HVA DA × 100 | 5-HIAA 5-HT × 100 |
|-------------|-------------------|-------------------------------|----------------|--------------|------------------|
|             |                   | DA | DOPAC | HVA | 5-HT | 5-HIAA |                  |              |                |
| Hippocampus | 0                 | 5  | 8.90 ± 0.32 | 1.33 ± 0.46 | 0.73 ± 0.026 | 0.33 ± 0.011 | 0.27 ± 0.019 | 82             |                |
|             | 5                 | 5  | 9.77 ± 0.27 | 1.45 ± 0.050 | 0.69 ± 0.049 | 0.38 ± 0.022 | 0.35 ± 0.018 | 92             |                |
|             | 20                | 5  | 9.33 ± 0.38 | 1.24 ± 0.081 | 0.72 ± 0.023 | 0.37 ± 0.017 | 0.38 ± 0.023³ | 103            |                |
|             | 20                | 1  | 9.61 ± 0.37 | 1.21 ± 0.033 | 0.59 ± 0.017³ | 0.37 ± 0.019 | 0.39 ± 0.022³ | 105            |                |
| Striatum    | 0                 | 5  | 8.90 ± 0.32 | 1.33 ± 0.46 | 0.73 ± 0.026 | 0.47 ± 0.009 | 0.43 ± 0.024 | 14.9           | 8.2            | 92              |
|             | 5                 | 5  | 9.77 ± 0.27 | 1.45 ± 0.050 | 0.69 ± 0.049 | 0.52 ± 0.026 | 0.46 ± 0.020 | 14.8           | 7.1            | 89              |
|             | 20                | 5  | 9.33 ± 0.38 | 1.24 ± 0.081 | 0.72 ± 0.023 | 0.49 ± 0.028 | 0.47 ± 0.026 | 13.3           | 7.7            | 96              |
|             | 20                | 1  | 9.61 ± 0.37 | 1.21 ± 0.033 | 0.59 ± 0.017³ | 0.51 ± 0.022 | 0.50 ± 0.029 | 12.6           | 6.1            | 98              |
| Cortex      | 0                 | 5  | 0.57 ± 0.11 | 0.100 ± 0.012 | 0.065 ± 0.008 | 0.31 ± 0.036 | 0.19 ± 0.007 | 17.5           | 11.4           | 61              |
|             | 5                 | 5  | 0.50 ± 0.10 | 0.079 ± 0.013 | 0.057 ± 0.010 | 0.31 ± 0.033 | 0.20 ± 0.018 | 15.8           | 11.4           | 65              |
|             | 20                | 5  | 0.35 ± 0.10 | 0.064 ± 0.007 | 0.053 ± 0.007 | 0.26 ± 0.015 | 0.19 ± 0.010 | 18.3           | 15.1           | 73              |
|             | 20                | 1  | 0.57 ± 0.11 | 0.099 ± 0.017 | 0.064 ± 0.008 | 0.37 ± 0.009 | 0.23 ± 0.018 | 17.4           | 11.2           | 62              |
| Thalamus-midbrain | 0       | 5  | 0.44 ± 0.036 | 0.084 ± 0.005 | 0.070 ± 0.041 | 0.45 ± 0.015 | 19.1           | 64             |
|             | 5                 | 5  | 0.44 ± 0.070 | 0.077 ± 0.007 | 0.072 ± 0.051 | 0.52 ± 0.030 | 17.5           | 72             |
|             | 20                | 5  | 0.54 ± 0.077 | 0.088 ± 0.008 | 0.075 ± 0.059 | 0.58 ± 0.033³ | 16.3           | 77             |
|             | 20                | 1  | 0.55 ± 0.130 | 0.085 ± 0.010 | 0.70 ± 0.035 | 0.54 ± 0.034 | 16.0           | 77             |

The last treatment was done 2 hour before killing. The values are means ± S.E. for five rats. *P < 0.05, **P < 0.01, significantly different from the control (two-tailed Dunnett's t-test).
## Table 2. Content of DA, 5-HT and their metabolites in brain sections obtained from rats treated with verapamil

| Doses mg/kg | Days of treatment | Tissue levels: μg/g wet weight | DOPAC × 100 | HVA × 100 | 5-HIAA × 100 |
|-------------|-------------------|-------------------------------|------------|----------|-------------|
|             |                   | DA | DOPAC | HVA | 5-HT | 5-HIAA | DA | DA | 5-HT |
| Hippocampus |                   |    |       |     |      |       |     |     |     |
|             | 0                  |  8.52 ± 0.24 | 0.37 ± 0.030 | 0.30 ± 0.022 | 81 |
|             | 5                  |  8.36 ± 0.33 | 0.40 ± 0.036 | 0.30 ± 0.006 | 75 |
|             | 10                 | 0.37 ± 0.022 | 0.32 ± 0.011 | 87 |
|             | 10                 | 0.34 ± 0.018 | 0.28 ± 0.011 | 82 |
| Striatum    |                   |     |       |     |      |       |     |     |     |
|             | 0                  |  8.52 ± 0.24 | 1.20 ± 0.084 | 0.69 ± 0.042 | 0.40 ± 0.027 | 0.37 ± 0.024 | 14.1 | 8.1 | 93 |
|             | 5                  |  8.36 ± 0.33 | 1.13 ± 0.057 | 0.64 ± 0.050 | 0.37 ± 0.022 | 0.36 ± 0.015 | 13.5 | 7.7 | 97 |
|             | 10                 | 0.37 ± 0.032 | 0.78 ± 0.062 | 0.42 ± 0.019 | 0.43 ± 0.032 | 14.0 | 9.4 | 102 |
|             | 10                 | 0.34 ± 0.014 | 0.76 ± 0.055 | 0.40 ± 0.022 | 0.41 ± 0.018 | 14.4 | 8.6 | 103 |
| Cortex      |                   |     |       |     |      |       |     |     |     |
|             | 0                  |  0.53 ± 0.061 | 0.104 ± 0.017 | 0.060 ± 0.004 | 0.28 ± 0.025 | 0.21 ± 0.006 | 19.6 | 11.3 | 75 |
|             | 5                  |  0.65 ± 0.054 | 0.113 ± 0.019 | 0.060 ± 0.005 | 0.27 ± 0.027 | 0.22 ± 0.018 | 17.4 | 9.2 | 82 |
|             | 10                 | 0.63 ± 0.059 | 0.125 ± 0.018 | 0.055 ± 0.004 | 0.33 ± 0.024 | 0.23 ± 0.018 | 19.8 | 8.7 | 70 |
|             | 10                 | 0.59 ± 0.066 | 0.116 ± 0.023 | 0.063 ± 0.005 | 0.31 ± 0.021 | 0.24 ± 0.023 | 19.7 | 10.7 | 77 |
| Thalamus-midbrain |         |     |       |     |      |       |     |     |     |
|             | 0                  |  0.48 ± 0.067 | 0.091 ± 0.014 | 0.64 ± 0.027 | 0.44 ± 0.014 | 19.0 | 69 |
|             | 5                  |  0.52 ± 0.061 | 0.111 ± 0.014 | 0.68 ± 0.027 | 0.52 ± 0.030 | 21.3 | 65 |
|             | 10                 | 0.56 ± 0.071 | 0.100 ± 0.014 | 0.75 ± 0.046 | 0.55 ± 0.027 | 17.9 | 73 |
|             | 10                 | 0.44 ± 0.058 | 0.077 ± 0.014 | 0.67 ± 0.030 | 0.49 ± 0.028 | 17.5 | 73 |

The last treatment was done 2 hours before killing. The values are means ± S.E. for five rats. *P < 0.05, significantly different from the control (two-tailed Dunnett's t-test).
### Table 3. Content of DA, 5-HT and their metabolites in brain sections obtained from rats treated with diltiazem

| Doses mg/kg | Days of treatment | Tissue levels: μg/g wet weight | DOPAC DA × 100 | HVA DA × 100 | 5-HIAA 5-HT × 100 |
|-------------|-------------------|-------------------------------|----------------|--------------|-------------------|
|             |                   | DA | DOPAC | HVA | 5-HT | 5-HIAA | DA | HVA | 5-HT |
| **Hippocampus** |                   |     |       |     |      |        |     |      |      |
| 0           | 5                 | 0.34 ± 0.026 | 0.31 ± 0.026 | 0.37 ± 0.017 | 0.37 ± 0.026 | 91 |
| 5           | 5                 | 0.39 ± 0.040 | 0.37 ± 0.022 | 0.37 ± 0.017 | 0.37 ± 0.026 | 100 |
| 20          | 5                 | 0.39 ± 0.014 | 0.35 ± 0.026 | 0.39 ± 0.014 | 0.35 ± 0.026 | 90 |
| **Striatum** |                   |     |       |     |      |        |     |      |      |
| 0           | 5                 | 8.47 ± 0.33 | 1.28 ± 0.104 | 0.72 ± 0.016 | 0.46 ± 0.041 | 0.41 ± 0.032 | 15.1 | 8.5 | 89 |
| 5           | 5                 | 8.40 ± 0.40 | 1.30 ± 0.113 | 0.72 ± 0.044 | 0.48 ± 0.029 | 0.46 ± 0.019 | 15.5 | 8.6 | 96 |
| 20          | 5                 | 7.97 ± 0.31 | 1.21 ± 0.080 | 0.73 ± 0.046 | 0.44 ± 0.030 | 0.44 ± 0.027 | 15.2 | 9.2 | 100 |
| 20          | 1                 | 8.37 ± 0.33 | 1.28 ± 0.069 | 0.81 ± 0.064 | 0.46 ± 0.016 | 0.42 ± 0.022 | 15.3 | 9.7 | 91 |
| **Cortex**  |                   |     |       |     |      |        |     |      |      |
| 0           | 5                 | 0.54 ± 0.046 | 0.096 ± 0.004 | 0.069 ± 0.003 | 0.34 ± 0.032 | 0.20 ± 0.006 | 17.8 | 12.8 | 59 |
| 5           | 5                 | 0.38 ± 0.055 | 0.071 ± 0.004 | 0.069 ± 0.005 | 0.32 ± 0.033 | 0.23 ± 0.014 | 18.7 | 18.2 | 72 |
| 20          | 5                 | 0.35 ± 0.092 | 0.055 ± 0.013 | 0.064 ± 0.013 | 0.30 ± 0.026 | 0.23 ± 0.006 | 15.7 | 18.3 | 77 |
| 20          | 1                 | 0.38 ± 0.074 | 0.078 ± 0.009 | 0.072 ± 0.006 | 0.32 ± 0.010 | 0.22 ± 0.009 | 20.5 | 18.9 | 69 |
| **Thalamus-midbrain** |             |     |       |     |      |        |     |      |      |
| 0           | 5                 | 0.41 ± 0.034 | 0.081 ± 0.010 | 0.63 ± 0.034 | 0.44 ± 0.027 | 19.8 | 70 |
| 5           | 5                 | 0.85 ± 0.095b | 0.156 ± 0.012b | 0.74 ± 0.022 | 0.56 ± 0.031b | 18.4 | 76 |
| 20          | 5                 | 0.58 ± 0.061 | 0.110 ± 0.010 | 0.73 ± 0.050 | 0.57 ± 0.032b | 19.0 | 78 |
| 20          | 1                 | 0.67 ± 0.113 | 0.108 ± 0.022 | 0.67 ± 0.047 | 0.50 ± 0.037 | 16.1 | 75 |

The last treatment was done 2 hours before killing. The values are means ± S.E. for five rats. *P < 0.05, **P < 0.01, significantly different from the control (two-tailed Dunnnet’s t-test).
the 5-HIAA levels only in the thalamus-midbrain. However, the increase of 5-HIAA/5-HT ratio was inconsistent in all the brain areas. Repeated administration of diltiazem induced changes in DOPAC in the cortex and induced changes in DA, DOPAC and 5-HIAA contents only in the thalamus-midbrain (Table 3). Moreover, in the brain areas of the repeatedly treated animals, the 5-HIAA/5-HT ratio was more elevated than in those of the once-treated rats. The decrease of the DOPAC/DA or HVA/DA ratios was uncertain in all the cases with the possible exception of the striatum obtained from rats once treated with nifedipine (Table 1).

Interaction between nifedipine and fasting
The data are shown in Table 4 with the ex-

| Hours of starvation | Doses mg/kg | Tissue levels: µg/g wet weight | 5-HIAA/5-HT × 100 |
|---------------------|------------|--------------------------------|-------------------|
|                     |            | NE                             | 5-HT              | 5-HIAA          |
| Hypothalamus        |            |                                |                   |
| 2                   | 0          | 2.66 ± 0.086                   | 1.05 ± 0.037      | 0.59 ± 0.028    | 56               |
| 2                   | 20         | 2.40 ± 0.082<sup>a</sup>       | 1.09 ± 0.040      | 0.67 ± 0.028<sup>a</sup> | 61               |
| 8                   | 0          | 2.16 ± 0.049<sup>d</sup>       | 0.94 ± 0.036<sup>c</sup> | 0.50 ± 0.020<sup>c</sup> | 54               |
| 8                   | 20         | 2.22 ± 0.078                   | 1.07 ± 0.038<sup>b</sup> | 0.70 ± 0.020<sup>b</sup> | 65               |
| Brainstem (pons + medulla) |    |                                |                   |
| 2                   | 0          | 0.66 ± 0.021                   | 0.72 ± 0.024      | 0.41 ± 0.027    | 57               |
| 2                   | 20         | 0.61 ± 0.030                   | 0.77 ± 0.024      | 0.48 ± 0.038    | 62               |
| 8                   | 0          | 0.60 ± 0.013                   | 0.71 ± 0.026      | 0.40 ± 0.018    | 56               |
| 8                   | 20         | 0.62 ± 0.042                   | 0.77 ± 0.029      | 0.53 ± 0.013<sup>b</sup> | 69               |
| Hippocampus         |            |                                |                   |
| 2                   | 0          | 0.33 ± 0.042                   | 0.34 ± 0.016      | 0.29 ± 0.014    | 85               |
| 2                   | 20         | 0.25 ± 0.024                   | 0.32 ± 0.030      | 0.38 ± 0.035<sup>a</sup> | 119              |
| 8                   | 0          | 0.27 ± 0.010                   | 0.32 ± 0.002      | 0.27 ± 0.016    | 84               |
| 8                   | 20         | 0.27 ± 0.008                   | 0.35 ± 0.009      | 0.40 ± 0.026<sup>b</sup> | 114              |
| Striatum            |            |                                |                   |
| 2                   | 0          | 0.35 ± 0.010                   | 0.48 ± 0.009      | 0.42 ± 0.011    | 88               |
| 2                   | 20         | 0.33 ± 0.017                   | 0.50 ± 0.024      | 0.45 ± 0.041    | 90               |
| 8                   | 0          | 0.34 ± 0.006                   | 0.47 ± 0.017      | 0.36 ± 0.024    | 77               |
| 8                   | 20         | 0.37 ± 0.016                   | 0.54 ± 0.039      | 0.55 ± 0.029<sup>b</sup> | 102              |
| Cortex              |            |                                |                   |
| 2                   | 0          | 0.28 ± 0.014                   | 0.33 ± 0.023      | 0.21 ± 0.014    | 64               |
| 2                   | 20         | 0.25 ± 0.015                   | 0.31 ± 0.031      | 0.23 ± 0.017    | 74               |
| 8                   | 0          | 0.27 ± 0.017                   | 0.31 ± 0.023      | 0.18 ± 0.011    | 58               |
| 8                   | 20         | 0.26 ± 0.012                   | 0.32 ± 0.012      | 0.26 ± 0.012<sup>b</sup> | 81               |
| Thalamus-midbrain   |            |                                |                   |
| 2                   | 0          | 0.69 ± 0.027                   | 0.71 ± 0.015      | 0.46 ± 0.029    | 65               |
| 2                   | 20         | 0.61 ± 0.023<sup>a</sup>       | 0.70 ± 0.055      | 0.54 ± 0.029    | 77               |
| 8                   | 0          | 0.56 ± 0.022<sup>d</sup>       | 0.62 ± 0.026      | 0.39 ± 0.026    | 63               |
| 8                   | 20         | 0.59 ± 0.022                   | 0.69 ± 0.025      | 0.62 ± 0.031<sup>b</sup> | 90               |

The treatments were done 2 hours before killing. <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01, significantly different from the respective control. <sup>c</sup>P < 0.05, <sup>d</sup>P < 0.01, significantly different from respective group of non-fasted rats.
ception of those regarding DA and its metabolites which remained unchanged.

As regards to NE, the interaction "nifedipine/fasting" resulted in statistically significant changes in both the hypothalamus (F1,16 = 4.48, P < 0.05) and thalamus-midbrain (F1,16 = 5.09, P < 0.05). In particular, fasting reduced the amine content while nifedipine only decreased NE in the rats not subjected to fasting.

The hypothalamic 5-HT level was lowered by fasting (F1,16 = 4.96, P < 0.05); in this case, nifedipine did increase the amine content of the hypothalamus (F1,16 = 6.59, P < 0.05). As regards to 5-HIAA, the interaction resulted in statistically significant changes in the hypothalamus (F1,16 = 6.52, P < 0.05), striatum (F1,16 = 7.94, P < 0.05), cortex (F1,16 = 5.11, P < 0.05), and thalamus-midbrain (F1,16 = 5.53, P < 0.05). In all the brain areas of the fasted rats, nifedipine increased the 5-HIAA levels, while it increased 5-HIAA levels in the hypothalamus and hippocampus of non-fasted rats only. On the other hand, fasting reduced the 5-HIAA levels in the hypothalamus alone. Therefore, the nifedipine induced increase of the 5-HIAA/5-HT ratio was greater in fasted rats than in non-fasted ones, except for in the hippocampus.

DISCUSSION

In our previous study (1), we concluded that blockade of VSCC with a single i.p. administration of nifedipine, verapamil or diltiazem (a) increased the 5-HIAA levels (and, in general, the signs of activation of serotonergic systems) in various brain areas; (b) produced signs of inhibition of the dopaminergic system by decreasing the DOPAC and HVA levels, which was most pronounced in the striatum; and (c) decreased the NE levels in some brain areas.

In comparison with nifedipine, longer time after the treatments and/or greater doses of verapamil and diltiazem were generally required to produce these effects. In the present study, while the greatest doses of each drug were avoided, the period of observation after the treatment lasted only two hours. These facts could explain some discrepancies between the present and the previous reports. In general, at the doses administered, all three drugs had similar effects on the serotonergic systems, with nifedipine being more active than verapamil and diltiazem. As regards to the effects on dopaminergic systems, only nifedipine was effective. Finally none of the three drugs affected the NE levels in any of the brain regions, even though the lowering of the amine content, at least by nifedipine, was expected. The present data, also provided evidence for differences between the effects of single or repeated administration of various calcium antagonists.

As regards to serotonergic systems, the nifedipine-induced effects were above all observed in the hippocampus and thalamus-midbrain, being more marked after repeated administration than after single injection in the thalamus-midbrain alone. Diltiazem induced similar effects but in the thalamus-midbrain alone. In this brain region, verapamil also induced the same effects, but they were smaller than those of diltiazem. This fact may be due to the low doses of verapamil that were chosen to avoid excessive effects on the cardiovascular system. For higher doses, these peripheral effects could produce indirect actions on the central nervous system, which can be as severe as marked depression or coma (1). In all cases, the data obtained showed that the effects induced by calcium antagonists on the serotonergic systems increased or, at least, remained unchanged after some days of administration.

The effects of diltiazem on dopaminergic systems appeared to be occasional, while verapamil was ineffective on these systems. The inhibiting effect of acutely administered nifedipine on the striatal dopaminergic system was substantially confirmed by the fall of the HVA levels and the fall of the DOPAC/DA and HVA/DA ratios (1). However, no changes of these ratios, DA and metabolites were ob-
served after repeated drug administration. These facts suggested that tolerance developed to the effect of nifedipine on the striatal dopaminergic system.

In general, the administration of calcium antagonists repeated for some days did not produce effects other than those produced by single administration. However, the effects on serotonergic systems became more marked, above all in the cerebral areas with the highest density of $[^3]$H-nitrendipine binding sites (15), i.e., the hippocampus and thalamus-midbrain. This fact, strongly suggesting the central origin of the effects, confirmed that their underlying mechanism consisted of VSCC blockade, together with the observation that all the three calcium antagonists showed similar effects. A similar confirmation for the effects on dopaminergic systems was lacking. Unfortunately, the present data did not supply any information about the previously observed results that VSCC antagonists decreased the brain NE levels. On the other hand, our previous study (1), unlike the present, was performed on rats treated with the calcium antagonists after 6-hr fasting. The starvation, besides modifying the functional state of the brain monoaminergic systems, could also change the bioavailability of the drugs. In fact, since food ingestion is known to increase splanchnic blood flow, the hepatic extraction and metabolism of the drugs could be decreased in fasted rats (16). Therefore, it might be hypothesized that the fall of NE levels could be produced by an interaction between drugs and starvation. The fourth experiment, however, did not support this hypothesis since fasting, lowering "per se" the NE levels in some brain areas, abolished the effects of nifedipine. On the contrary, nifedipine did lower the NE levels in these areas when administered to non-fasted rats. Therefore, it is likely that in the first three experiments, some unknown factors have inadvertently affected the general procedure; thus they may have lowered the NE content of the various brain areas so that nifedipine was ineffective in further lowering the amine content. However, these considerations suggested that nifedipine would or would not change the NE levels in the brain areas depending on the functional state of the adrenergic system. From the fourth experiment, a similar speculation could be made about the effects of nifedipine on the serotonergic systems. In fact, fasting reduced "per se" the 5-HT and 5-HIAA levels in the hypothalamus alone, but potentiated the effects of nifedipine in all the brain areas except for the hippocampus. In particular, the nifedipine induced increases of the 5-HIAA levels and the 5-HIAA/5-HT ratios were much greater in the fasted rats than in the non-fasted ones. This finding agreed with the ability of nifedipine to reverse the decrease of 5-HIAA during morphine withdrawal (17).

In conclusion, nifedipine appeared to affect the adrenergic and/or serotonergic systems to a different extent depending on the functional state of these systems. This fact could be of great interest in view of the possible use of calcium antagonists to treat disorders of the central nervous system (18). It is likely that the properties of nifedipine could be extended, at least partly, to other calcium antagonists selective for the VSCC. In all cases, the present data and the pharmacokinetic properties of nifedipine (19, 20) suggested that this compound should be more suitable than verapamil or diltiazem for producing relatively selective actions on the central nervous system. On the other hand, the differences in the binding sites of the various subtypes of calcium antagonists on the macromolecular complex of VSCC, conditioning their specificity for the various brain areas, could determine the usefulness of each compound for treating specific disorders of the central nervous system.

REFERENCES

1 Gaggi, R. and Gianni, A.M.: Effects of calcium antagonists on biogenic amines in discrete brain areas. Eur. J. Pharmacol. 181, 187–197 (1990)
2 Bartholini, G. and Lloyd, K.G.: Biochemical effects of neuroleptic drugs. In Psychotropic Agents, Part I: Antipsychotics and Antidepres-
Calcium Antagonists and Brain Amines

sants. Edited by Hoffmister, H. and Stille, G., Vol. 55/I, p. 193–212, Springer-Verlag, Berlin Heidelberg and New York (1980)

3 Paoletti, R. and Govoni, S.: Classification of calcium antagonists: proposal of the WHO committee. Pharmacol. Res. Commun. 19, 195–208 (1987)

4 Vanhoutte, P.M. and Paoletti, R.: The WHO classification of calcium antagonists. Trends Pharmacol. Sci 8, 4–6 (1987)

5 Godfraind, T., Miller, R. and Wibo, M.: Calcium antagonism and calcium entry blockade. Pharmacol. Rev. 38, 321–416 (1986)

6 De Vries, D.J. and Beart, P.M.: Competitive inhibition of [3H]piperone binding to D-2 dopamine receptors in striatal homogenates by calcium channel antagonists and polyvalent cations. Eur. J. Pharmacol. 106, 133–139 (1985)

7 Swanson, T.H. and Green, C.L.: Nifedipine: more than a calcium channel blocker. Gen. Pharmacol. 17, 255–260 (1986)

8 De Feudis, F.V.: Interaction of Ca2+ antagonists at 5-HT2 and H1 receptor and GABA uptake sites. Trends Pharmacol. Sci. 8, 200–202 (1987)

9 Zernig, G.: Widening potential for Ca2+ antagonists: non-L-type Ca2+ channel interaction. Trends Pharmacol. Sci. 11, 38–44 (1990)

10 Miller, R.J.: Multiple calcium channels and neuronal functions. Science 235, 46–52 (1987)

11 Middlemiss, D.N. and Spedding, M.: A functional correlate for the dihydropyridine binding site in rat brain. Nature 314, 94–96 (1985)

12 Wagner, J., Vitali, P., Palfreyman, M.G., Zraika, M. and Huot, S.: Simultaneous determination of 3,4-dihydroxyphenylalanine, 5-hydroxytryptophan, dopamine 4-hydroxy-3-methoxyphenylalanine, norepinephrine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, serotonin and 5-hydroxyindoleacetic acid in rat cerebrospinal fluid and brain by high-performance liquid chromatography with electrochemical detection. J. Neurochem. 38, 1241–1254 (1982)

13 Seegal, R.F., Brosh, K.O. and Bush, B.: High-performance liquid chromatography of biogenic amines and metabolites in brain, cerebrospinal fluid, urine and plasma. J. Chromatogr. 377, 131–144 (1986)

14 Gaggi, R., De Iasio, R. and Gianni, A.M.: Relationships between the effects of peripherally administered salmon calcitonin on calcaemia and brain biogenic amines. Japan. J. Pharmacol. 51, 309–320 (1989)

15 Gould, R.J., Murphy, K.M.M and Snyder, S.H.: Autoradiographic localization of calcium channel antagonist receptors in rat brain with [3H]-nitrendipine. Brain Res. 330, 217–223 (1985)

16 Welling, P.G.: Effects of food on drug absorption. Pharmacol. Ther. 43, 425–441 (1989)

17 Colado, M.I., Lorenzo, P. and Martin, M.I.: Nifedipine reversal of decreased serotonin metabolite levels during morphine withdrawal. Arch. Int. Pharmacodyn. Ther. 298, 61–67 (1989)

18 Raeburn, D. and Gonzales, R.A.: CNS disorders and calcium antagonists. Trends Pharmacol. Sci. 9, 117–119 (1988)

19 Sorkin, E.M., Clissold, S.P. and Brodgen, R.N.: Nifedipine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy, in ischemic heart disease, hypertension and related cardiovascular disorders. Drugs 30, 182–274 (1985)

20 Janicki, P.K., Siembab, D., Paulo, E.A. and Krzasek, P.: Single-dose kinetics of nifedipine in rat plasma and brain. Pharmacology 36, 183–187 (1988)