Effects of Butorphanol and Its Metabolites on the Levels of Monoamines and Their Metabolites in the Rat Brain

Masayuki NIWA, Takashi NOSE, Masakatsu NOZAKI, Kaito TSURUMI and Hajime FUJIMURA*

Department of Pharmacology, Gifu University School of Medicine, Tsukasa 40, Gifu 500, Japan
*Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607, Japan

Accepted September 2, 1985

Abstract—The effects of butorphanol and its main metabolites, norbutorphanol and hydroxybutorphanol, on the contents of monoamines and their metabolites in various regions of the rat brain were compared with those of morphine and pentazocine using the HPLC-ECD method. The administrations of morphine and pentazocine increased dopamine turnover in the striatum and hypothalamus in a drug dose-dependent manner. The stimulative effects of butorphanol on the dopamine system were weaker than those of morphine and pentazocine, and there were no dose-dependencies in these effects of butorphanol. Butorphanol, morphine and pentazocine increased 5-HT turnover, but there was no drug dose-dependent effect in the case of butorphanol. These differences for the effects of butorphanol from those of morphine and pentazocine seemed to result from the antagonist-agonist property of butorphanol and from a different manner of interaction with the opioid receptor. The effects of butorphanol on the levels of the norepinephrine system were weak. It was considered that the effects of butorphanol on monoamine turnover were produced by the action of butorphanol itself, because norbutorphanol and hydroxybutorphanol showed little change on the level of monoamines and their metabolites.

Butorphanol (BT) possesses potent analgesic properties with a moderate narcotic antagonistic activity (1) and weak opioid dependent activities (2, 3). BT has both μ-antagonist and δ-agonist-like properties against opioid receptors (4). It is well known that opiate compounds change amounts of norepinephrine (NE), dopamine (DA) and 5-hydroxytryptamine (5-HT) in the brain via the opioid receptors (5–7). The change of the levels of NE and 5-HT is responsible for analgesic activities of opioids, and the modification of dopaminergic activity is closely related to the alterations of mental and behavioral states.

It has been reported that BT changed the concentrations of DA, its metabolites and NE in whole rat brain (8). BT has at least two main metabolites (9), norbutorphanol (NB) which showed mild analgesic activity and hydroxybutorphanol (HB) which possessed weak narcotic antagonist property (3). We have suggested that these activities of NB and HB are ascribed to the μ-agonistic property and the δ-antagonistic property, respectively (4).

The neurochemical effects of antagonist-agonist have not been well defined compared with morphine and other opioid agonists. Therefore, the present paper dealt with the acute effects of BT, a representative antagonist-agonist, and BT metabolites on the levels of monoamines and their metabolites compared with morphine and pentazocine in various regions of rat brain.

Materials and Methods

Chemicals: BT, NB and HB were supplied by Bristol Banyu Mfg. Co., Ltd. Morphine hydrochloride (Mor, Takeda) and pentazocine hydrochloride (PZ, a gift from Dr. Hori of the Gifu Pharmaceutical University) were used as
the reference drugs. Dopamine hydrochloride was obtained from Nakarai Chemical Co. and 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), norepinephrine hydrochloride, 3,4-dihydroxybenzylamine hydrobromide (DHBA) and 5-HT creatinine sulfate were purchased from Sigma. 5-Hydroxyindole-3-acetic acid (5-HIAA) and 3,4-dihydroxyhydrocinnamic acid (DOPPA) were obtained from Aldrich.

Animals: Wistar strain male rats weighing 200–270 g (Kitayama Labs) were used. The rats were maintained in an air-conditioned room (22±2°C), and pellet feed (Oriental Yeast, MF) and tap water were supplied ad libitum. Seven to eight rats and 1–3 rats were used for the groups for drug treatments and control, respectively.

Administration of drugs: BT, NB, HB, Mor and PZ were dissolved in distilled water and 2 ml/kg of each solution was subcutaneously injected into the back of the animal. The control group, distilled water was injected in the same manner. BT was administered at 0.5, 5 and 50 mg/kg, and the dose of NB or HB was 50 mg/kg. PZ was injected at 5 and 50 mg/kg. Mor was given at 5 and 25 mg/kg, because of toxic effects at 50 mg/kg. All of drugs were administered at 9:00–11:00 a.m.

Extraction of amines and metabolites from brain: Brain enzymes were rapidly inactivated by the microwave irradiation (5.0 KW, 1.1 sec) using a metabostat system (Shin Nippon Musen, NJE 2601) at 1 hr after administration of the drugs in all groups. Then, the brain was immediately isolated and cooled on ice. Each brain was dissected into seven areas, the cortex, hippocampus, striatum, hypothalamus, midbrain, pons-medulla oblongata and cerebellum according to the method of Glowinski and Iversen (10). Extraction was performed according to the modified method of Nabeshima et al. (11). Briefly, each tissue from brain was homogenized by a glass homogenizer using a medium consisting of 2 ml ice cooled n-butanol, 50 μl of 50 mM EDTA and 250 μl of 0.025 N hydrochloric acid containing internal standards for HPLC (DHBA 200 ng, DOPPA 150 ng). The homogenate was saturated with NaCl by adding 1 g NaCl and was shaken for 60 min at 15°C. The mixture was then centrifuged at 1600×g for 10 min at 4°C. One ml of butanol layer was transferred into the centrifuge tube containing 2 ml n-heptane and 120 μl of 0.1 N hydrochloric acid and was shaken for 10 min at 15°C. Subsequently, the mixture was centrifuged again at 1600×g for 10 min at 4°C. The aqueous layer was stored in a deep freezer at −80°C to use for simultaneous assay of monoamines.

In order to extract metabolites of monoamines, 100 μl of 0.2 M Tris-HCl buffer (pH 8.5) containing 0.01% ascorbic acid, was added to 2.5 ml of the organic layer, and the mixture was shaken for 1 min. Then the sample was centrifuged at 1600×g for 5 min at 4°C. The aqueous layer was stored at −80°C for assay of monoamine metabolites.

Determination of monoamines and metabolites: A high-performance liquid chromatography system with an electrochemical detector (Irika Industries, P-321 and E308) was used, and a 25 cm reverse phase C-18 (Irika Industries, RP-18S or Nomura Chemical, DEVELOSIL ODS-5) was used for separation. Monoamines and their metabolites were determined by applying 10 μl of extracted solution. A mobile phase consisting of 0.1 M citric acid buffer (pH 4.5) containing 0.5 mM 1-heptanesulfonic acid sodium salt mono-hydrate (Aldrich) and 1.5% tetrahydrofuran (THF, Wako) was used for simultaneous determinations of monoamines, and 0.08 M citric acid buffer (pH 3.9) containing 1% THF, 6% acetic acid (Wako) and 10% ethanol (Wako) were used for assay of their metabolites. The flow rate of the mobile phase was 0.5 ml/min, and the oxidation potential for the detector was set at 0.8 V vs. Ag/AgCl.

The peak height was measured for determination by the internal standard method.

Statistical evaluations of experimental data were performed by the analysis of variance in a completely randomized design with repetition.

The recovery rates of NE, both DA and DOPAC, 5-HIAA and both 5-HT and HVA were about 60%, 70%, 80% and 90% or more, respectively.
Results

Effects of BT on the level of DA and its metabolites: The effects of BT, Mor and PZ on the total amounts of DA and its metabolites, DOPAC and HVA, in various brain regions are summarized in Table 1. Figure 1 shows the percent changes of the concentrations of DA, DOPAC and HVA in the striatum and hypothalamus by treatment with the test drugs.

As we reported previously (8), the contents of DOPAC and HVA in whole brain significantly increase in a drug dose-dependent manner after administration of Mor or PZ, and the treatment with PZ decreases the level of DA in correlation with the increases of the contents of DOPAC and HVA. In contrast, BT shows few effects on the levels of DOPAC, HVA and DA.

The treatment with Mor increased the DOPAC and HVA levels in the striatum and three regions of the brain stem (hypothalamus, midbrain and pons-medulla oblongata) in a drug dose-dependent manner. In the striatum and hypothalamus, the magnitude of increase of the DOPAC level was the same as that of the HVA level. Similar results were obtained in the case of the treatment with PZ. PZ elicited a significant increase in the DOPAC and HVA levels in the striatum and hypothalamus, and the increments of these levels after the administration of 50 mg/kg PZ were larger than those after 25 mg/kg Mor injection. PZ increased the HVA levels in the cortex, hippocampus and cerebellum. The DA levels in the striatum, hypothalamus, midbrain and hippocampus decreased by treatment with PZ, and the most remarkable change was observed for the hypothalamus.

By treating with BT, the DOPAC and HVA levels in the striatum and hypothalamus slightly increased, but there was no dose-dependency. The maximum effects of BT on these levels were obtained in the group of 5 mg/kg dose. BT increased the HVA level in the midbrain. In the pons-medulla oblongata, the HVA level was not affected, and the DOPAC level showed a little decrease even at a dose of 50 mg/kg BT. The DA levels in the striatum and three regions of the brain stem were not influenced by BT. The levels of HVA and DA in the cortex significantly increased without dose-dependency by the administration of BT.

It is well known that the cerebellum is defective of DA neurone. However, we were able to detect a trace amount of DA and its metabolites in the cerebellum, the same reported by others (12–14).

Effects on NE system: Table 2 shows the effects of BT, Mor and PZ on NE contents in various brain regions. The NE levels in the cortex, hippocampus, hypothalamus and pons-medulla oblongata slightly increased by the administration of Mor. A drug dose-dependency of the change of NE level was observed only for the hypothalamus. The treatment with 25 mg/kg Mor decreased the NE level in the midbrain.

The administration of 5 mg/kg PZ significantly increased the NE levels in the cortex, hippocampus and striatum. However these changes were not of significance in the group of 50 mg/kg dose, and the NE levels in the hypothalamus and midbrain decreased.

Treating with BT, the NE levels in the hippocampus, hypothalamus and pons-medulla oblongata slightly increased without a drug dose-dependency. As a preliminary experiment, the content of a free form of MHPG, which is a metabolite of NE, was determined in the pons-medulla oblongata. The MHPG level for the untreated control group was 38.0±4.6 ng/g wet tissue. The administration of 25 mg/kg Mor significantly increased the MHPG level, that is 78.1±8.4 ng/g wet tissue. In contrast, the MHPG levels after the administration of BT at 0.5, 5 and 50 mg/kg dose were 31.7±4.7, 44.2±11.2, and 44.3±9.0 ng/g wet tissue, respectively, showing no significant difference from the level of the control group.

Effects on 5-HT system: The effects of BT, Mor and PZ on the levels of 5-HT and its metabolite, 5-HIAA, in various brain regions are summarized in Table 3. Figure 2 shows the effects of these three drugs on 5-HT and 5-HIAA levels in three regions of the brain stem.

The administration of Mor increased the 5-HT level in each brain region except for the striatum. Especially, the levels in the hypothalamus and pons-medulla oblongata sig-
Table 1. Effects of butorphanol, morphine and pentazocine on the content of dopamine and its metabolites in rat brain

| Brain site    | Drug     | Dose (mg/kg) | Dopamine     | DOPAC | HVA   |
|---------------|----------|--------------|--------------|-------|-------|
|               |          |              | (ng/g wet tissue) |       |       |
| Control       | Butorphanol | 0.5          | 23.9 ± 3.2 (7) | 7.8 ± 1.0 (7) | 10.8 ± 1.3 (6) |
|               |          |              | 5            | 61.5 ± 13.5* (6) | 13.1 ± 1.7* (5) | 26.5 ± 5.6** (5) |
|               |          |              | 50           | 51.4 ± 24.5 (3) | 11.7 ± 3.6 (3) | 25.1 ± 7.2* (3) |
|               |          |              | Morphine     | 62.0 ± 24.6* (6) | 14.2 ± 3.0 (4) | 26.4 ± 7.0* (4) |
|               |          |              | 50           | 26.9 ± 9.4 (3) | 11.9 ± 4.7 (3) | 11.9 ± 3.2 (3) |
|               |          |              | 5            | 30.5 ± 10.8 (4) | 10.4 ± 3.0 (3) | 18.2 ± 4.2 (3) |
|               |          |              | 50           | 26.0 ± 3.0 (4) | 6.8 ± 1.6 (3) | 13.5 ± 2.5 (3) |
|               | Pentazocine | 5            | 30.2 ± 9.5 (4) | 12.4 ± 6.0 (3) | 19.8 ± 2.1 (3) |
|               | Butorphanol | 0.5          | 66.4 ± 15.5 (9) | 22.4 ± 6.5 (7) | 18.5 ± 7.4 (7) |
|               |          |              | 5            | 59.0 ± 7.5 (7) | 13.5 ± 1.7 (6) | 17.8 ± 2.9 (6) |
|               |          |              | 50           | 81.5 ± 25.9 (6) | 16.2 ± 4.3 (5) | 21.9 ± 4.9 (5) |
|               | Morphine  | 5            | 55.4 ± 6.9 (6) | 13.7 ± 1.2 (4) | 16.3 ± 2.2 (4) |
|               |          |              | 25           | 70.7 ± 6.0 (4) | 19.3 ± 3.1 (3) | 22.6 ± 4.6 (3) |
|               |          |              | 50           | 56.4 ± 6.2 (4) | 17.4 ± 3.3 (3) | 25.6 ± 6.6 (3) |
|               | Pentazocine | 5            | 54.9 ± 14.0 (4) | 13.9 ± 1.7 (3) | 21.9 ± 0.7 (3) |
|               |          |              | 50           | 52.4 ± 10.3 (4) | 18.1 ± 3.9 (3) | 28.5 ± 8.7 (3) |
| Control       | Butorphanol | 0.5          | 8940.2 ± 502.2 (13) | 856.2 ± 60.4 (8) | 809.8 ± 81.9 (8) |
|               |          |              | 5            | 9452.9 ± 1063.4 (9) | 1082.2 ± 127.8 (6) | 1015.9 ± 38.4 (6) |
|               |          |              | 50           | 9191.4 ± 672.0 (8) | 1010.9 ± 76.9 (5) | 1073.4 ± 53.1 (5) |
|               | Morphine  | 5            | 9172.5 ± 878.4 (8) | 1048.1 ± 187.1 (5) | 1019.6 ± 90.0 (5) |
|               |          |              | 25           | 9593.8 ± 1465.3 (6) | 1337.3 ± 240.8* (3) | 1338.0 ± 255.8** (3) |
|               |          |              | 50           | 9600.7 ± 999.6 (7) | 1506.6 ± 137.0** (4) | 1350.0 ± 241.6** (4) |
|               | Pentazocine | 5            | 9325.5 ± 1057.2 (7) | 937.3 ± 91.2 (4) | 936.4 ± 81.6 (4) |
|               |          |              | 50           | 7062.7 ± 903.2 (7) | 1728.6 ± 222.4*** (4) | 1576.7 ± 233.9*** (4) |
| Control       | Butorphanol | 0.5          | 420.9 ± 46.7 (11) | 81.3 ± 7.8 (7) | 59.5 ± 9.6 (7) |
|               |          |              | 5            | 353.9 ± 32.9 (7) | 89.3 ± 2.9 (4) | 75.2 ± 7.6 (4) |
|               |          |              | 50           | 447.1 ± 57.8 (6) | 106.0 ± 17.4 (4) | 91.3 ± 14.8 (4) |
|               | Hypothalamus | 5            | 360.2 ± 43.5 (6) | 84.1 ± 10.3 (4) | 71.1 ± 12.0 (4) |
|               | Morphine  | 5            | 433.5 ± 87.9 (5) | 120.4 ± 23.9 (3) | 88.8 ± 12.1 (3) |
|               |          |              | 25           | 502.3 ± 45.3 (5) | 161.1 ± 11.7** (4) | 111.7 ± 20.6* (4) |
|               |          |              | 50           | 351.5 ± 38.0 (5) | 106.1 ± 10.4 (3) | 91.5 ± 16.7 (3) |
|               | Pentazocine | 5            | 264.7 ± 39.6* (5) | 181.7 ± 50.3*** (3) | 122.3 ± 33.3** (3) |
| Brain site                  | Drug    | Dose (mg/kg) | Dopamine | DOPAC | HVA   |
|----------------------------|---------|--------------|----------|-------|-------|
|                            |         |              | Content (ng/g wet tissue) |       |       |
|                            | Control | 169.0±12.1 (11) | 45.5±2.9 (7) | 52.0±5.1 (7) |
|                            | Butorphanol | 174.8±11.8 (7) | 44.7±1.4 (5) | 72.4±2.9 (5) |
|                            | 5       | 189.9±27.9 (6) | 56.8±7.3 (4) | 80.7±10.9 (4) |
| Midbrain                   | 50      | 168.1±23.9 (6) | 35.7±2.4 (4) | 65.1±7.2 (4) |
|                            | Morphine | 182.6±23.9 (5) | 48.8±15.8 (3) | 86.5±24.0* (3) |
|                            | 25      | 193.0±10.3 (5) | 61.5±2.9* (4) | 100.6±18.7*** (4) |
|                            | Pentazocine | 165.7±17.3 (5) | 38.8±7.5 (3) | 77.3±15.3 (3) |
|                            | 50      | 125.6±13.1 (5) | 59.1±7.4 (3) | 95.3±12.0* (3) |
|                            | Control | 54.7±5.3 (11) | 34.8±4.0 (8) | 38.8±4.8 (8) |
|                            | Butorphanol | 63.9±3.8 (7) | 26.3±3.6 (5) | 41.8±8.3 (5) |
|                            | 5       | 57.3±3.4 (6) | 28.2±2.4 (4) | 41.4±6.1 (4) |
| Pons and medulla oblongata | 50      | 52.0±3.5 (6) | 21.5±3.4* (4) | 33.1±4.8 (4) |
|                            | Morphine | 59.1±7.1 (5) | 33.4±2.1 (3) | 51.0±7.8 (3) |
|                            | 25      | 79.0±5.9** (5) | 41.5±0.9 (4) | 72.2±11.9** (4) |
|                            | Pentazocine | 52.5±7.0 (5) | 31.2±3.0 (4) | 49.6±3.7 (4) |
|                            | 50      | 55.1±7.2 (5) | 46.0±6.6* (4) | 64.9±8.3** (4) |
|                            | Control | 9.3±3.5 (7) | 9.7±1.1 (8) | 14.2±1.6 (8) |
|                            | Butorphanol | 8.8±2.1 (4) | 8.0±0.7 (6) | 19.6±2.3 (6) |
|                            | 5       | 12.5±3.3 (3) | 10.0±1.3 (5) | 14.1±1.1 (5) |
| Cerebellum                 | 50      | 8.4±1.3 (4) | 10.4±4.0 (4) | 13.5±1.8 (4) |
|                            | Morphine | 8.7±1.1 (3) | 15.0±6.1 (3) | 27.5±9.4* (3) |
|                            | 25      | 8.2±0.1 (3) | 12.1±2.4 (3) | 25.2±1.1* (3) |
|                            | Pentazocine | 7.7±1.2 (4) | 11.3±3.5 (4) | 18.6±2.6 (4) |
|                            | 50      | 8.1±2.7 (3) | 11.8±2.7 (3) | 29.7±10.8** (3) |

Each data value represents the mean±S.E. and numbers of observations per group are indicated in parentheses. *, ** and *** indicate significant differences from the control at P<0.05, P<0.01 and P<0.001, respectively.
The levels of 5-HT in the striatum tended to decrease. There was a clear dose-dependency in these changes of 5-HT levels. The 5-HIAA levels increased in all regions of brain, but only significantly in the hypothalamus.

PZ caused a change of 5-HT level in each region of the brain in a manner similar to Mor. Treating with PZ, 5-HT levels in the cortex, midbrain and pons-medulla oblongata increased or tended to increase in a drug dose-dependent manner. The 5-HIAA levels tended to increase in most of the brain regions.

On the 5-HT levels in brain regions, increases or increasing tendencies were observed, except in the striatum by the administration of BT, without a drug dose-dependency. The maximum effects of BT significantly increased at a dose of 25 mg/kg Mor. The levels of 5-HT in the striatum tended to decrease. There was a clear dose-dependency in these changes of 5-HT levels. The 5-HIAA levels increased in all regions of brain, but only significantly in the hypothalamus.

PZ caused a change of 5-HT level in each region of the brain in a manner similar to Mor. Treating with PZ, 5-HT levels in the cortex, midbrain and pons-medulla oblongata increased or tended to increase in a drug dose-dependent manner. The 5-HIAA levels tended to increase in most of the brain regions.

On the 5-HT levels in brain regions, increases or increasing tendencies were observed, except in the striatum by the administration of BT, without a drug dose-dependency. The maximum effects of BT
Table 2. Effects of butorphanol, morphine and pentazocine on norepinephrine content in rat brain

| Drug      | Dose (mg/kg) | Cortex   | Hippocampus | Striatum | Hypothalamus | Midbrain | Pons and Medulla oblongata | Cerebellum |
|-----------|--------------|----------|-------------|----------|--------------|----------|---------------------------|------------|
| Control   |              | 224.4±17.9 (8) | 317.3±38.6 (7) | 90.0± 8.3 (7) | 945.3± 86.8 (11) | 523.4±31.0 (11) | 517.5±41.1 (11) | 262.7±31.7 (10) |
| Butorphanol | 0.5          | 254.4±34.0 (4) | 380.9±29.9 (6) | 93.7±17.7 (3) | 971.7±130.2 (7) | 523.1±60.4 (7) | 585.5±35.1 (7) | 286.9±44.4 (6) |
|           | 5            | 272.2±44.8 (3) | 433.4±68.6 (5) | 79.0±19.8 (3) | 1158.9±219.9 (6) | 512.6±87.9 (6) | 546.4±67.2 (6) | 257.1±46.1 (5) |
|           | 50           | 225.5±39.1 (5) | 339.4±32.8 (5) | 103.1±13.1 (3) | 1004.0±198.5 (6) | 516.7±84.7 (6) | 564.4±59.4 (6) | 273.7±65.9 (5) |
| Morphine  | 5            | 310.9±54.2 (3) | 402.3±96.8 (3) | 60.7± 4.1 (3) | 973.7±276.8 (5) | 574.0±97.6 (5) | 574.4±89.5 (5) | 309.8±54.5 (4) |
|           | 25           | 274.7±36.3 (3) | 397.9±51.2 (3) | 77.8± 4.7 (3) | 1199.8±102.5 (5) | 500.7±67.9 (5) | 561.6±55.4 (6) | 277.4±40.9 (4) |
| Pentazocine | 5            | 339.1±68.4* (3) | 494.2±82.8* (3) | 138.7±38.3* (4) | 1025.8±127.5 (5) | 584.6±65.6 (5) | 584.0±77.6 (5) | 317.6±58.5 (5) |
|           | 50           | 226.2±23.4 (4) | 417.8±99.4 (3) | 87.8±13.2 (4) | 871.9±182.3 (5) | 449.7±63.3 (5) | 541.8±82.5 (5) | 259.2±57.2 (4) |

Each data value represents the mean±S.E., and numbers of observations per group are indicated in parentheses. *, indicates significant difference from the control at P<0.05.
Table 3. Effects of butorphanol, morphine and pentazocine on the content of 5-HT and its metabolites in rat brain

| Brain site | Drug       | Dose (mg/kg) | Content (ng/g wet tissue) | 5-HT | 5-HIAA |
|------------|------------|--------------|--------------------------|------|--------|
|            |            |              |                          |      |        |
|            | Control    |              |                          |      |        |
|            | Butorphanol| 0.5          | 336.1 ± 22.2 (8)         |      | 243.9 ± 16.5 (7) |
|            |            | 5            | 526.3 ± 53.3* (6)       |      | 365.0 ± 10.6 (5) |
|            |            | 10           | 520.8 ± 36.8* (3)       |      | 341.5 ± 47.0 (3) |
| Cortex     |            | 25           | 495.1 ± 70.2* (6)       |      | 323.4 ± 31.1 (4) |
|            | Morphine   | 5            | 606.8 ± 77.6 (3)        |      | 412.3 ± 153.6 (3) |
|            |            | 25           | 435.3 ± 71.0 (4)        |      | 392.4 ± 64.0 (3) |
|            | Pentazocine| 5            | 501.5 ± 76.7* (4)       |      | 338.0 ± 132.7 (3) |
|            |            | 50           | 543.9 ± 62.8* (4)       |      | 320.0 ± 77.8 (3) |
|            | Control    |              |                          |      |        |
|            | Butorphanol| 0.5          | 401.5 ± 49.8 (9)        |      | 694.1 ± 65.6 (7) |
|            |            | 5            | 566.2 ± 55.8 (7)        |      | 725.7 ± 83.6 (6) |
|            | 10          | 535.7 ± 57.9 (6) |                  |      | 693.8 ± 162.1 (5) |
|            | 25          | 521.5 ± 46.9 (6) |                  |      | 679.9 ± 92.2 (4) |
| Hippocampus| Morphine   | 5            | 637.7 ± 119.0* (4)      |      | 795.4 ± 217.2 (3) |
|            |            | 25           | 415.9 ± 76.6 (4)        |      | 767.0 ± 41.6 (3) |
|            | Pentazocine| 5            | 771.6 ± 177.3*** (4)    |      | 690.2 ± 206.2 (3) |
|            |            | 50           | 471.5 ± 136.3 (4)       |      | 625.3 ± 165.7 (3) |
|            | Control    |              |                          |      |        |
|            | Butorphanol| 0.5          | 507.8 ± 43.1 (13)       |      | 718.0 ± 33.6 (8) |
|            |            | 5            | 526.2 ± 45.1 (8)        |      | 741.9 ± 28.1 (6) |
|            | 10          | 453.8 ± 57.3 (8) |                  |      | 774.9 ± 45.1 (5) |
| Striatum   | Morphine   | 5            | 423.7 ± 68.3 (8)        |      | 606.9 ± 62.6 (5) |
|            |            | 25           | 490.7 ± 61.0 (6)        |      | 798.3 ± 183.9 (3) |
|            | Pentazocine| 5            | 436.5 ± 39.5 (7)        |      | 919.3 ± 105.8 (4) |
|            |            | 50           | 577.4 ± 41.7 (7)        |      | 841.3 ± 125.7 (4) |
|            | Control    |              |                          |      |        |
|            | Butorphanol| 0.5          | 890.3 ± 30.9 (11)       |      | 885.6 ± 57.1 (7) |
|            |            | 5            | 1059.5 ± 78.3* (7)      |      | 1111.2 ± 102.1 (4) |
|            | 10          | 1148.1 ± 63.4** (6) |              |      | 1112.3 ± 264.6 (4) |
| Hypothalamus| Morphine  | 5            | 1043.6 ± 78.8* (6)      |      | 818.4 ± 71.9 (4) |
|            |            | 25           | 996.5 ± 55.1 (5)        |      | 1131.2 ± 202.0 (3) |
|            | Pentazocine| 5            | 1218.5 ± 51.0** (5)     |      | 1261.4 ± 57.9* (4) |
|            |            | 50           | 1001.6 ± 28.9 (5)       |      | 1088.0 ± 35.7 (3) |
|            | Control    |              |                          |      |        |
|            | Butorphanol| 0.5          | 1110.0 ± 126.3 (11)     |      | 1224.2 ± 118.9 (7) |
|            |            | 5            | 1337.8 ± 182.7 (7)      |      | 1396.4 ± 58.9 (5) |
|            | 10          | 1270.3 ± 81.7 (6) |              |      | 1413.5 ± 248.7 (4) |
| Midbrain   | Morphine   | 5            | 1265.3 ± 106.8 (6)      |      | 1108.3 ± 123.7 (4) |
|            |            | 25           | 1310.2 ± 115.5 (5)      |      | 1605.9 ± 364.8 (3) |
|            | Pentazocine| 5            | 1242.7 ± 66.2 (5)       |      | 1448.4 ± 113.1 (4) |
|            |            | 50           | 1265.4 ± 107.5 (5)      |      | 1519.6 ± 97.2 (3) |
|            | Control    |              |                          |      |        |
|            | Butorphanol| 0.5          | 717.3 ± 69.6 (11)       |      | 830.7 ± 47.9 (8) |
|            |            | 5            | 1057.8 ± 106.9*** (7)   |      | 882.0 ± 146.6 (5) |
| Pons and   | Morphine   | 5            | 917.5 ± 51.9* (6)       |      | 929.8 ± 227.6 (4) |
| medulla oblongata | | 50     | 870.7 ± 42.3 (6)        |      | 698.5 ± 92.3 (4) |
|            | Pentazocine| 5            | 828.9 ± 98.1 (5)        |      | 989.1 ± 286.9 (3) |
|            |            | 25           | 941.9 ± 32.2* (6)       |      | 1038.5 ± 66.0 (4) |
|            |            | 50           | 784.0 ± 72.7 (5)        |      | 880.9 ± 78.8 (4) |
|            | Control    |              |                          |      |        |
|            | Butorphanol| 0.5          | 806.9 ± 52.1 (5)        |      | 920.3 ± 118.9 (4) |
were observed in the cortex (P<0.01), hippocampus, midbrain and pons-medulla oblongata (P<0.001) at a dose of 0.5 mg/kg and in the hypothalamus (P<0.01) at a dose of 5 mg/kg. However, the 5-HT level in the striatum decreased in a similar manner to that in the Mor-treated groups. In most of the brain regions, the 5-HIAA levels tended to increase at doses of 0.5 and 5 mg/kg, except at 50 mg/kg.

Effects of BT-metabolites (NB and HB) on brain monoamine levels: The results are summarized in Table 4. The brain monoamine levels were little affected in almost of the regions by the treatment with HB. NB produced significant increases of both levels of the HVA and 5-HIAA in the cortex, and the HVA level in midbrain. HB decreased the NE contents in the cortex and cerebellum. BT exhibits an agonistic activity at low doses and exerts predominantly an antagonistic activity at high doses (16). PZ, a narcotic antagonist-agonist, increased the turnover rate of DA in a drug dose-dependent manner, suggesting that the antagonistic activity should be weak (22).

Discussion
It has been reported that opiates including opioid peptides enhance the metabolic turnover rate of the DA system, resulting in increases of the DOPAC and HVA levels in the striatum (15–17), and Mor stimulates the DA biosynthesis in the hypothalamus and striatum (18). The modifications of the DA system by opioids are known to be mediated via the opioid receptors (19–21).

The administration of Mor and PZ increased the DOPAC and HVA levels in some regions of rat brain such as the striatum and hypothalamus, suggesting that these drugs stimulate the rate of metabolic conversion of the DA system. The effects of BT on the DA system were weaker than those of Mor and PZ, and there were no drug dose-dependencies for the effects of BT. The most marked increases in levels of DA metabolites were obtained after administration of the lower dose of BT in the striatum and hypothalamus. BT possesses μ-antagonist and κ-agonist-like properties against opioid receptors (4). Wood et al. have described that κ-agonists have little influence on the amount of DA metabolites in the striatum and that narcotic antagonist-agonist affected the levels of DA metabolites in the striatum with bell-shaped dose-response curves (16, 19). BT exhibits an agonistic activity at low doses and exerts predominantly an antagonistic activity at high doses (16). PZ, a narcotic antagonist-agonist, increased the turnover rate of DA in a drug dose-dependent manner, suggesting that the antagonistic activity should be weak (22).

Generally, the cataleptogenic property and DA metabolites increasing property in the striatum of an opioid agonist run parallel (17, 23, 24). It is reported that PZ causes little catalepsy, in spite of its DA metabolites increasing property (24). BT showed no activity to cause catalepsy in the toxicological studies (25, 26). In our experiment, catalepsy was observed by administration of Mor, but not PZ and BT. Wood et al. (16) reported that both antagonist-agonist and κ-agonist caused no catalepsy. The non-cataleptogenic property of BT may be ascribed in antagonist-
agonistic and κ-agonist like activity.

It has been reported that Mor had no influences on the turnover of NE (18, 27). However, recent many reports (28–30) demonstrated that Mor increased MHPG-SO₄, a major metabolite of NE, and that Mor decreased NE in the whole brain or some brain regions. These effects were antagonized by naloxone (28, 30). In the present experiment, Mor and BT caused no changes of the NE concentration in any brain regions. However, in the preliminary experiment, Mor increased the content of MHPG, intermediary metabolite from NE to MHPG-SO₄, but BT showed no activity in the pons-medulla oblongata. Therefore, the mode of action of BT on the NE system may be different from that of Mor.

The opioids stimulate the turnover of 5-HT in the brain, resulting in an increase of the 5-HIAA level (5, 7, 31). This effect was antagonized by pretreatment with naloxone.
Table 4. Effects of norbutorphanol and hydroxybutorphanol on the content of brain monoamines and their metabolites in rat

|                | Dopamine | DOPAC | HVA   | Norepinephrine | 5-HT     | 5-HIAA   |
|----------------|----------|-------|-------|----------------|----------|----------|
| **Cortex**     |          |       |       |                |          |          |
| Control        | 31.5 ± 7.1(9) | 7.8 ± 0.9(9) | 14.2 ± 2.6(8) | 224.5 ± 14.5(10) | 360.6 ± 24.0(10) | 268.7 ± 20.7(9) |
| nor-BT         | 42.3 ± 18.9(4) | 12.5 ± 3.1(5) | 40.0 ± 12.7*(5) | 211.5 ± 28.3(5) | 358.6 ± 43.3(5) | 358.0 ± 18.8***(5) |
| OH-BT          | 48.6 ± 3.8(5) | 11.2 ± 1.9(5) | 27.8 ± 4.8(5) | 165.7 ± 14.6*(5) | 288.3 ± 33.2(5) | 306.2 ± 15.6(5) |
| **Hypocampus** |          |       |       |                |          |          |
| Control        | 61.0 ± 13.0(11) | 20.2 ± 5.2(9) | 17.8 ± 5.7(9) | 315.3 ± 29.8(9) | 426.5 ± 45.0(11) | 632.4 ± 58.2(9) |
| nor-BT         | 72.5 ± 30.1(5) | 13.4 ± 2.7(5) | 19.8 ± 1.8(5) | 275.0 ± 36.4(5) | 466.6 ± 33.4(5) | 753.2 ± 80.0(5) |
| OH-BT          | 32.3 ± 3.9(5) | 12.4 ± 2.1(5) | 18.7 ± 2.5(5) | 256.4 ± 11.7(5) | 413.0 ± 45.5(5) | 723.1 ± 48.5(5) |
| **Striatum**   |          |       |       |                |          |          |
| Control        | 9267.0 ± 501.8(15) | 920.3 ± 82.5(10) | 855.2 ± 84.2(10) | 91.4 ± 6.9(9) | 506.5 ± 37.3(15) | 732.9 ± 34.9(10) |
| nor-BT         | 9111.6 ± 1264.1(5) | 1018.6 ± 182.0(5) | 1024.8 ± 72.8(5) | 90.9 ± 14.4(5) | 475.9 ± 51.6(5) | 775.4 ± 59.9(5) |
| OH-BT          | 9308.8 ± 843.2(5) | 952.6 ± 151.1(5) | 997.5 ± 69.3(5) | 85.6 ± 10.6(5) | 499.0 ± 32.9(5) | 747.6 ± 41.0(5) |
| **Hypothalamus** |         |       |       |                |          |          |
| Control        | 380.1 ± 48.1(13) | 72.7 ± 8.3(9) | 55.9 ± 7.9(9) | 889.3 ± 83.4(13) | 847.3 ± 39.8(13) | 926.0 ± 63.7(9) |
| nor-BT         | 393.8 ± 48.4(4) | 85.2 ± 12.2(5) | 69.4 ± 8.8(5) | 969.2 ± 118.4(4) | 846.4 ± 45.9(4) | 1019.7 ± 96.3(5) |
| OH-BT          | 304.5 ± 24.2(5) | 71.4 ± 8.0(5) | 53.1 ± 5.3(5) | 916.5 ± 117.9(5) | 763.6 ± 51.8(5) | 932.3 ± 71.5(5) |
| **Midbrain**   |          |       |       |                |          |          |
| Control        | 158.8 ± 12.3(13) | 42.1 ± 3.3(9) | 51.0 ± 4.3(9) | 507.3 ± 29.0(13) | 1089.5 ± 107.7(13) | 1213.8 ± 95.6(9) |
| nor-BT         | 203.5 ± 25.6(4) | 51.2 ± 4.0(5) | 71.6 ± 3.6***(5) | 474.1 ± 44.7(4) | 1146.9 ± 97.3(4) | 1266.2 ± 125.9(5) |
| OH-BT          | 183.3 ± 15.0(5) | 45.5 ± 6.8(5) | 61.3 ± 6.3(5) | 417.6 ± 20.4(5) | 885.3 ± 30.3(5) | 1087.6 ± 72.6(5) |
| **Pons and medulla oblongata** |         |       |       |                |          |          |
| Control        | 55.5 ± 4.5(13) | 32.9 ± 3.5(10) | 39.6 ± 4.1(10) | 516.0 ± 34.5(13) | 729.3 ± 59.0(13) | 843.7 ± 48.9(10) |
| nor-BT         | 61.0 ± 6.8(5) | 27.1 ± 4.5(5) | 43.3 ± 5.7(5) | 518.7 ± 70.9(5) | 736.5 ± 103.6(5) | 819.0 ± 142.1(5) |
| OH-BT          | 62.3 ± 8.6(5) | 24.5 ± 2.8(5) | 38.8 ± 4.9(5) | 518.7 ± 68.0(5) | 686.1 ± 86.1(5) | 761.0 ± 90.5(5) |
| **Cerebellum** |          |       |       |                |          |          |
| Control        | 9.7 ± 2.8(9) | 9.2 ± 1.0(10) | 15.1 ± 1.7(10) | 268.9 ± 28.3(12) | 175.9 ± 21.8(12) | 176.1 ± 14.1(10) |
| nor-BT         | 5.0 ± 0.4(4) | 6.3 ± 0.6(4) | 15.5 ± 1.4(4) | 178.2 ± 4.7(4) | 158.0 ± 15.0(4) | 144.5 ± 2.4(4) |
| OH-BT          | 5.1 ± 0.8(4) | 8.8 ± 1.1(5) | 16.7 ± 1.8(5) | 166.4 ± 28.6*(4) | 162.7 ± 47.5(4) | 160.7 ± 19.9(5) |

Each data value represents the mean ± S.E. and number of observations per group are indicated in parentheses. Control: saline s.c., nor-BT: norbutorphanol 50 mg/kg, s.c., OH-BT: hydroxybutorphanol 50 mg/kg, s.c. * and ** indicate differences from the control at P < 0.05 and P < 0.01, respectively.
PZ is reported to possess no stimulative activity on the metabolic turnover of the 5-HT system in the whole brain (6).

BT and PZ, like Mor, elicited increase or increasing tendency to the 5-HT and 5-HIAA levels in most regions of the brain. The analgesic activities of BT and Mor are markedly diminished by the treatment of 5-HT depleting agents, but that of PZ is not affected at all. Therefore, BT has a stimulative activity on the turnover of the 5-HT system, and the analgesic action of BT can be ascribed in part to manifestation through the 5-HT system. The effects of PZ on the 5-HT system in the hypothalamus and pons-medulla oblongata were the weakest among the tested drugs. These results suggested that the mechanism of analgesic action of PZ is somewhat different from those of Mor and BT.

In the hypothalamus and pons-medulla oblongata, BT showed an increase of the 5-HT level at the dose of 0.5 or 5 mg/kg, and this activity decreased at the dose of 50 mg/kg BT. Similar tendencies were obtained on the 5-HIAA levels. These phenomena were not observed in the case of the treatments of Mor and PZ. BT exhibits a ceiling effect for analgesic activities (33). The analgesic activities of BT are recognized at closer lower than 0.5 mg/kg using the tail pinch method and the acetic acid writhing method in mice, and the activity is not potentiated any more even at doses higher than 2 mg/kg. There were good relationships between the pattern of the effects of BT on the 5-HT system in the brain stem and that of the analgesic activities of BT. The narcotic antagonist agonist may be characterized with these phenomena.

NB and HB have been found to be major metabolites of BT, and NB exhibits a mild analgesic activity, and HB possesses antagonistic activities to pharmacological actions of narcotics (3, 5, 7), which may be contributed to \( \mu \)-agonist and \( \mu \)-antagonistic activities of NB and HB, respectively (4). At only a high dose (50 mg/kg) of NB, the contents of the HVA and 5-HIAA in the cortex decreased, being responsible for an interaction with the opioid receptor. HB decreased the NE levels in the cortex and cerebellum. It is not clear whether the antagonistic activity of HB is attributable to these changes. The effects of BT on the brain monoamine levels were produced by the action of BT itself, because there were few effects of NB and HB in spite of their large quantities of administration.

The mode of the stimulative action of BT on the metabolic turnover in 5-HT and DA systems is different from those of Mor and PZ. The effects of BT showed a poor drug dose-dependency. BT exhibited milder effects to the turnover in the NE system than Mor. The receptor interaction of BT is suggested to be different from those of Mor and PZ.

References
1 Pircio, A.W., Gylys, J.A., Cavanagh, R.L., Buyinski, J.P. and Bierwagen, M.E.: The pharmacology of butorphanol, a 3, 14-dihydroxy-morphinan narcotic antagonist analgesic. Arch. Int. Pharmacodyn. Ther. 220, 231–257 (1976)
2 Snyder, S.H.: Clinical relevance of opiate receptor and opioid peptide research. Nature 279, 13–14 (1979)
3 Niwa, M., Nozaki, M., Kamikubo, K., Fujimura, H., Kadota, T., Kai, S., Kawano, S., Kohmura, H. and Takahashi, N.: The physical dependence liabilities of butorphanol, a narcotic antagonist, and its main metabolites, norbutorphanol and hydroxybutorphanol. Folia Pharmacol. Japon. 82, 451–463 (1983) (Abs. in English)
4 Nozaki, M., Niwa, M., Hasegawa, J. and Fujimura, H.: Opioid receptor interactions of butorphanol, a narcotic antagonist analgesic, and its metabolites. Folia Pharmacol. Japon. 82, 443–450 (1983) (Abs. in English)
5 Garcia-Sevilla, J.A., Ahtee, L., Magnusson, T. and Carlsson, A.: Opiate-receptor mediated changes in monoamine synthesis in rat brain. J. Pharm. Pharmacol. 30, 613–621 (1978)
6 Goodlet, I. and Sugrue, M.F.: Effect of acutely administered analgesic drugs on rat brain serotonin turnover. Eur. J. Pharmacol. 29, 241–248 (1974)
7 Yarbrough, G.G., Buxbaum, D.M. and Sanders-Bush, E.: Biogenic amines and narcotic effects. II. Serotonin turnover in the rat after acute and chronic morphine administration. J. Pharmacol. Exp. Ther. 185, 328–335 (1973)
8 Niwa, M., Nozaki, M., Fujimura, H., Nose, T. and Takahashi, N.: Effects of butorphanol tartrate, a narcotic antagonist analgesic, on the concent-
trations of monoamines and their metabolites in rat whole brain. Iyakuhin Kenkyu 15, 82–86 (1984)

9 Heel, R.C., Brogden, R.N., Speight, T.M. and Avery, G.S.: Butorphanol: A review of its pharmacological properties and therapeutic efficacy. Drugs 16, 473–505 (1978)

10 Glowinski, J. and Iversen, L.L.: Regional studies of catecholamines in the rat brain—I. The disposition of 3H-norepinephrine, 3H-dopamine and 3H-dopa in various regions of the brain. J. Neurochem. 13, 655–669 (1966)

11 Nabeshima, T., Hiramatsu, M., Noma, S., Ukai, M., Amano, M. and Kameyama, T.: Determination of methionine-enkephalin, norepinephrine, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylacetic acid (HVA) in brain by high-pressure liquid chromatography with electrochemical detector. Res. Commun. Pathol. Pharmacol. 35, 421–442 (1982)

12 Izumi, K., Motomatsu, T., Chretien, M., Buterworth, R.F., Lis, M., Seidah, N. and Barbeau, A.: β-Endorphin induced akinesia in rats: Effect of apomorphine and α-methyl-p-tyrosine and related modifications of dopamine turnover in the basal ganglia. Life Sci. 20, 1149–1156 (1977)

13 Mefford, I.N., Gilberg, M. and Barchas, J.D.: Simultaneous determination of catecholamines and unconjugated 3,4-dihydroxyphenylacetic acid in brain tissue by ion-pairing reverse-phase high-performance liquid chromatography with electrochemical detection. Anal. Biochem. 104, 469–472 (1980)

14 Maruyama, Y. and Kusaka, M.: Assay of norepinephrine and dopamine in the rat brain after microwave irradiation. Life Sci. 23, 1603–1608 (1978)

15 McMillen, B.A.: On the mechanism of morphine action on rat striatal dopamine metabolism. Biochem. Pharmacol. 29, 1432–1435 (1980)

16 Wood, P.L., Stotland, M., Richard, J.W. and Rackham, A.: Actions of mu, kappa, sigma, delta and agonist/antagonist opiates on striatal dopaminergic function. J. Pharmacol. Exp. Ther. 215, 697–703 (1980)

17 Van Loon, G.R. and Kim, C.: β-Endorphin-induced increase in striatal dopamine turnover. Life Sci. 23, 961–970 (1978)

18 Clouet, D.H. and Ratner, M.: Catecholamine biosynthesis in brains of rats treated with morphine. Science 168, 854–856 (1970)

19 Wood, P.L., Sanschagrin, D., Richard, J.W. and Thakur, M.: Multiple opiate receptor affinities of kappa and agonist/antagonist analgesics: In vivo assessment. J. Pharmacol. Exp. Ther. 226, 545–550 (1983)

20 Gomes, C., Svensson, T.H. and Trolin, G.: Effects of morphine on central catecholamine turnover, blood pressure and heart rate in the rat. Naunyn Schmiedebergs Arch. Pharmacol. 294, 141–147 (1976)

21 Pollard, H., Llorens-Cortes, C. and Schwartz, J.C.: Enkephalin receptors on dopaminergic neurones in rat striatum. Nature 268, 745–747 (1977)

22 Harris, L.S. and Pierson, A.K.: Some narcotic antagonists in the benzomorphan series. J. Pharmacol. Exp. Ther. 143, 141–148 (1964)

23 Kuschinsky, K. and Hornykiewicz, O.: Morphine catalepsy in the rat: Relation to striatal dopamine metabolism. Eur. J. Pharmacol. 19, 119–122 (1972)

24 Ahtee, L. and Käariainen, I.: The effect of narcotic analgesics on the homovanillic acid content of rat nucleus caudatus. Eur. J. Pharmacol. 22, 206–208 (1973)

25 Takahashi, N., Kadota, T., Kawano, S. and Ishikawa, K.: Toxicological studies of butorphanol tartrate. (I) Acute toxicity in mouse and rat. Iyakuhin Kenkyu 13, 145–152 (1982)

26 Henmi, Z., Mitsushima, T., Araki, K., Kawazura, H., Saitoh, S., Horigome, K., Ozeki, K., Kamiya, J., Nishina, T., Yoshiwara, K., Ishii, M., Okada, N., Mizuchi, A. and Tokuda, H.: General pharmacological effects of butorphanol. Japan. Pharmacol. Ther. 12, 55–82 (1984)

27 Papieschi, R., Theiss, P. and Herz, A.: Effects of morphine on the turnover of brain catecholamines and serotonine in rats acute morphine administration. Eur. J. Pharmacol. 34, 253–261 (1975)

28 Lopachin, R.M. and Reigle, T.G.: The effects of several narcotic analgesics on brain levels of 3-methoxy-4-hydroxyphenylethylamine sulfate in the rat. J. Pharmacol. Exp. Ther. 207, 151–158 (1978)

29 Roffman, M., Reigle, H., Orsulak, P.; Cassens, G. and Schildkraut, J.J.: Further studies of the effects of morphine on the levels of 3-methoxy-4-hydroxyphenylethylamine sulfate in rat brain. Neuropharmacology 18, 483–488 (1979)

30 Tanaka, M., Kohno, Y., Tsuda, A., Nakagawa, R., Ida, Y., Limori, K., Hoaki, Y. and Nagasaki, N.: Differential effects of morphine on noradrenaline release in brain regions of stressed and nonstressed rats. Brain Res. 275, 105–116 (1983)

31 Larson, A.A. and Takemori, A.E.: Effect of morphine on the fate of newly transported tryptophan and 5-hydroxytryptophan in brain of
rats. J. Pharmacol. Exp. Ther. 205, 265–273 (1978)

32 Mitsushima, T., Saitoh, S., Kawazura, H. and Henmi, Z.: Effects of monoamine related drug on analgesic effect of butorphanol. Japan. Pharmacol. Ther. 12, 31–36 (1984)

33 Kawazura, H., Saitoh, S., Araki, K., Mitsushima, T. and Henmi, Y.: Analgesic effect of butorphanol. Japan. Pharmacol. Ther. 12, 13–24 (1984)