1) (*12*). Thus, cells in the amygdaloid complex seem to provide the caudate with some of its CCK, but which amygdaloid cells do so remains to be determined.

In cats the caudate nucleus has been reported to receive projections from the claustrum, although the claustrum sends rather few of its axons there (*13*). Immunocytochemical studies of this structure and of the adjacent piriform cortex revealed heavy staining for CCK in both areas (Fig. 1A). In the claustrum, CCK was principally in axons, but scattered cell bodies were also stained (Fig. 1B). In the piriform cortex CCK was found mainly in cell bodies.

A knife cut severing the claustrum and the piriform cortex from the caudatoputamen (*14*) decreased the CCK concentration in the caudatoputamen by approximately 70 percent (Table 1). This knife cut did not compromise the blood supply to the caudate and caused a minimal amount of nonspecific, mechanical damage. The perikarya that stain for CCK in the piriform cortex are situated adjacent to the claustrum. Selective ablation of the piriform cortex was therefore impossible. Consequently, we could not determine whether the caudatoputamen receives its CCK-containing afferents from the claustrum, the piriform cortex, or both.

The claustrum receives afferents from various cortical areas whereas the piriform cortex is part of the olfactory system (*15*). Thus CCK might be the transmitter in the final common pathway linking various cortical areas or the olfactory system to the caudatoputamen. This finding suggests possible new directions for research on the basal ganglia and raises the possibility that CCK-containing neurons in the claustrum-piriform cortex, which provide most of the CCK found in the caudatoputamen, may be involved in regulating the caudatoputamen.

D. K. MEYER
M. C. BEINFELD
W. H. OERTEL
M. J. BROWNSTEIN

Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland 20205

References and Notes

1. O. Hornykiewicz, *Adv. Exp. Med. Biol.* **90**, 1 (1977); R. Elde, T. Hokfelt, O. Johansson, L. Terenius, *Neuroscience* **1**, 349 (1976); A. C. Cicello, I. Kanazawa, *J. Comp. Neurol.* **178**, 129 (1978); G. R. Uhl, M. J. Kuhar, S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4059 (1977); F. M. A. Correa, R. B. Innis, G. R. Uhl, S. H. Snyder, *ibid.* **76**, 1489 (1979).
2. O. Hornykiewicz, in *The Basal Ganglia*, M. D. Yahr, Ed. (Raven, New York, 1976), p. 269; K. G. Lloyd, in *Essays in Neurochemistry and Neuropharmacology*, M. B. Youdim *et al.*, Eds. (Wiley, New York, 1978), vol. 3, p. 131.
3. L. I. Larsson and J. F. Rehfeld, *Brain Res.* **165**, 201 (1979); M. C. Beinfeld, D. K. Meyer, R. L. Eskay, R. T. Jensen, M. J. Brownstein, *ibid.* **212**, 51 (1981).
4. A. Saito, H. Sankaran, I. D. Goldfine, J. A. Williams, *Science* **208**, 1155 (1980); S. E. Hays, D. K. Meyer, S. M. Paul, *Brain Res.*, in press; S. E. Hayes, M. C. Beinfeld, R. T. Jensen, F. K. Goodwin, S. M. Paul, *Neuropeptides* **1**, 53 (1980).
5. R. B. Innis, F. M. A. Correa, G. R. Uhl, B. Schneider, S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 521 (1979); I. Loren, J. Alumets, R. Hakanson, F. Sundler, *Histochemistry* **59**, 249 (1979); J. J. Vanderhaegen, F. Lotstra, J. de Mey, C. Gilles, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1190 (1980).
6. H. J. W. Nauta, M. B. Pritz, R. J. Lasek, *Brain Res.* **67**, 219 (1974); J. G. Veenig, F. M. Cornelissen, P. A. J. M. Lieven, *Neuroscience* **5**, 253 (1980).
7. A 2-mm blade was inserted 4.3 mm anterior to the interauricular line down to the bone, with the medial edge of the blade in the midline (plane angle 11° down). The rats weighed 200 to 250 g.
8. Dopamine concentrations were determined as described by R. M. McCarty, G. Gilad, V. K. Weise, and I. J. Kopin [*Life Sci.* **25**, 747 (1979)]. The dopamine concentration was 0.87 ± 0.07 μg per gram, wet weight (mean ± standard error; $N = 8$) in the unlesioned side and not measurable in the lesioned side.
9. The dopamine and CCK content of the nucleus accumbens was also measured in rats with unilateral lesions in the median forebrain bundle. The dopamine content was reduced 90 percent by this lesion whereas the CCK content was unchanged indicating that the mesencephalic regions such as the ventral tegmental area are not the source of the bulk of the CCK in the nucleus accumbens. See T. Hokfelt, L. Skirbol, J. F. Rehfeld, M. Goldstein, K. Marhey, and O. Dann [*Neuroscience* **5**, 2093 (1980)] for a description of the codistribution of dopamine and CCK.
10. Separation was achieved with a 5-mm blade inserted 11.0 mm anterior to the interauricular line down to bone; the angle of the blade to midline was 45°, the middle edge of the blade was 4.0 mm lateral to the midline, and the plane angle was 11° down.
11. After removing a bone flap covering the dorsal part of the brain, the accessible cortical areas were removed by suction.
12. The cut was made with a 3-mm blade, 7.5 mm anterior to the interauricular line down to bone, with the middle edge of the blade 2 mm lateral to the midline, and the plane angle 11° down.
13. R. Druga, *Folia Morphol. (Prague)* **20**, 163 (1972).
14. The cut was made with a 6-mm blade, 11.0 mm anterior to the interauricular line down to bone, with the angle of the blade to midline being 45°, the middle edge of the blade 2.6 mm lateral to the midline, and the plane angle 11° down.
15. J. B. Carman, W. M. Cowan, T. P. S. Powell, *J. Neurol. Neurosurg. Psychiatry* **27**, 46 (1964); R. Druga, *Folia Morphol. (Prague)* **16**, 142 (1968); D. Sanides and C. S. Buchholtz, *Exp. Brain Res.* **35**, 197 (1979); T. P. S. Powell, W. M. Cown, G. Raisman, *J. Anat.* **99**, 791 (1965); L. B. Haberly and J. L. Price, *J. Comp. Neurol.* **178**, 711 (1978).
16. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
17. L. A. Sternberger, *Immunocytochemistry* (Wiley, New York, ed. 2, 1979).

5 August 1981; revised 30 October 1981

Substance P Analog, DiMe-C7: Evidence for Stability in Rat Brain and Prolonged Central Actions

Abstract. *A metabolically protected analog of substance P, [pGlu^5-MePhe^8-MeGly^9]SP(5–11) (DiMe-C7), was approximately equipotent with substance P in causing increased locomotor activity after microinfusion into the ventral tegmental area of rat brain, but the effects of DiMe-C7 on behavior were considerably prolonged. There was little metabolic degradation of tritiated DiMe-C7 for up to 1 hour after infusion, whereas tritiated substance P was completely degraded within 10 minutes.*

Substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) (SP) occurs in neurons in many parts of the mammalian central nervous system and is widely regarded as a putative neurotransmitter (*1–3*). Assessing the central actions of SP, however, is made difficult by the metabolic instability of this peptide in brain (*4*). We have recently synthesized an analog of SP ([pGlu^5-MePhe^8-MeGly^9]SP(5–11) (DiMe-C7) that is resistant to attack by a substance P–degrading enzyme purified from brain (*5*) and is metabolically stable when incubated with rat brain hypothalamic slices or synaptosomal membrane preparations in vitro (*5*, *6*). The analog competes with ^{3}H-labeled SP for binding to receptor sites in rat brain membranes and also retains biological activity in various peripheral test systems, although its potency ranges from 1/10 to 1/50 of that of SP (*6*, *7*).

We now present evidence that DiMe-C7 is metabolically stable when administered directly to rat brain and that it produces persistent behavioral responses. To assess the central actions of the analog we used a behavioral model previously developed in this laboratory, in which substance P or related compounds are microinfused in the region of dopamine cell groups in rat brain (*8*, *9*). When SP is locally administered into the ventral tegmental area (VTA) or substantia nigra it causes characteristic patterns of hyperactivity (*8*, *9*). Accordingly, we infused SP and DiMe-C7 into the rat VTA and determined the time course of the behavioral response. In parallel experiments with radioactive peptides (*10*) we examined the time course of disappearance of unchanged peptides after they were locally microinfused into the VTA.

Peptides were administered through stereotaxically implanted guide cannulas aimed at the VTA, and locomotor responses were recorded in photocell ac-

0036-8075/82/0108-0188$01.00/0

Fig. 1. Mean locomotor activity responses after infusing equal doses of substance P (*SP*) and DiMe-C7 (in saline) into rat VTA. The rats were infused bilaterally with (A) SP (2.5 μg in 2 μl of saline) (N = 8), DiMe-C7 (2.5 μg/2 μl) (N = 7), or 2 μl of saline (N = 7), and (B) SP (5 μg/2 μl) (N = 8), DiMe-C7 (5 μg/2μl)) (N = 7), or 2 μl of saline (N = 7). Results of Tukey comparisons: *, $P < .001$ relative to substance P–treated rats and controls; ++, $P < .001$ and +, $P < .01$ relative to controls only.

tivity cages (*8*, *9*, *11*). In experiments in which [^{3}H]SP and [^{3}H]DiMe-C7 were used, animals were killed at various time intervals after injection of the labeled peptides, the brains were rapidly removed and chilled, and the VTA, substantia nigra, and other brain areas were dissected and stored frozen. Radioactive compounds were subsequently extracted for counting, and the unchanged peptides separated from radioactive metabolites by thin-layer chromatography.

When equal doses of SP and DiMe-C7 were administered into the VTA the resulting locomotor responses had different time courses (Fig. 1A). After administration of 2.5-μg doses, the initial responses to SP and DiMe-C7 were not significantly different, but the effect of DiMe-C7 was significantly prolonged when compared with the transient response to SP. A similarly prolonged response occurred after administration of 5 μg of DiMe-C7, although in this case the initial response was also significantly greater than that after injection of an equal dose of SP (Fig. 1B). These results indicate that DiMe-C7 is approximately equipotent with SP in its effects at receptors in rat brain, but its actions are considerably prolonged. This is in accord with our previous findings that indicated that DiMe-C7 had a similar potency to SP itself in competing for [^{3}H]SP binding to receptor sites in rat brain membrane preparations in vitro (*6*).

When [^{3}H]SP was microinfused into the VTA there was a rapid metabolic breakdown and disappearance of the labeled peptide. Indeed, we were unable to detect unchanged [^{3}H]SP in the VTA or any other brain region 10 minutes after injection and the labeled SP accounted for only 13 percent of the radioactivity present in the VTA 5 minutes after injection (Fig. 2A). A complex mixture of labeled peptide fragments was present in VTA, but using high-performance liquid chromatography analyses we failed to find any of the known biologically active fragments of this peptide. In contrast, after injections of [^{3}H]DiMe-C7 there was no detectable metabolic degradation

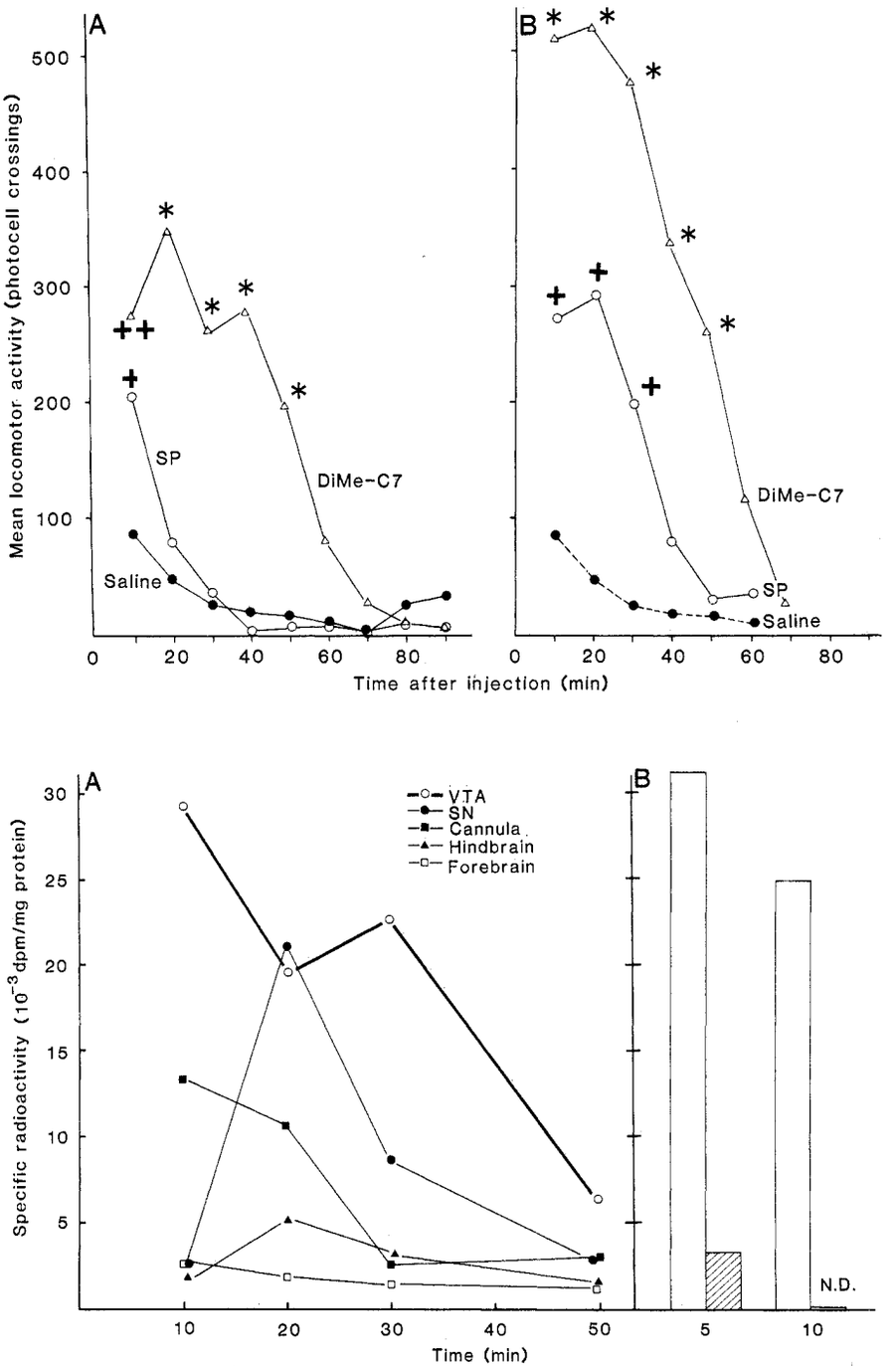

Fig. 2. Disappearance of (A) ^{3}H-labeled DiMe-C7 and (B) [^{3}H]-labeled SP after microinfusion into the VTA of rat brain. In each animal, 2.5 μg of peptide (corresponding to 8.38 μCi for [^{3}H]DiMe-C7 and 6.95 μCi for [^{3}H]SP) was infused bilaterally. Dissected brain regions were extracted by means of a Teflon-glass homogenizer in 10 volumes of boiling 1*M* acetic acid (5 minutes), and duplicate 10-μl portions were taken for Lowry protein analysis. The homogenates were centrifuged for 5 minutes at 10,000*g* to remove debris and were freeze-dried. For [^{3}H]SP, the dried samples were dissolved in 50 percent acetic acid and portions were chromatographed as described (*5*). For [^{3}H]DiMe-C7, dried samples were extracted in ethanol and portions were chromatographed on LQD silica gel plates with a mixture of *n*-propanol and water (7:3 by volume). Tritium-labeled compounds were detected by a Berthold radiochromatogram scanner and unchanged peptides were identified by comparison with radioactive and nonradioactive standards. Duplicate portions were counted directly by liquid scintillation to determine total radioactivity. Peaks corresponding in R_F to unchanged peptides were extracted from the plates and counted. In (A), no degradation was detected until the 50-minute time point, and values reported are the means of determinations on three separate animals for the unchanged [^{3}H]DiMe-C7. Abbreviations: *VTA*, ventral tegmental area and *SN*, substantia nigra. In (B), the open bars show the total radioactivity of [^{3}H]SP and metabolites, and the hatched bar shows the proportion of unchanged [^{3}H]SP in the extract. At the 10-minute time point no unchanged [^{3}H]SP could be detected (N.D., none detected). Values reported are the means of three separate animals.

during the first 30 minutes and only a small proportion (< 20 percent) of the radioactivity remaining after 50 minutes could be attributed to radioactively labeled metabolites. The unchanged peptide disappeared from the focal infusion site with a time course similar to that observed for the behavioral response after DiMe-C7 administration (Fig. 2B). The disappearance of DiMe-C7, however, appears largely to be due to diffusion away from the injection site rather than metabolic degradation, because unchanged peptide appeared in the adjacent substantia nigra and in hindbrain with a time delay of about 20 minutes after injection of DiMe-C7 into the VTA (Fig. 2B). The delayed appearance of DiMe-C7 in adjacent brain areas may help to explain various additional behavioral responses that occur after administration of this analog into the VTA (*12*).

These results support the suggestion that DiMe-C7 represents a metabolically stable and biologically active analog of SP in the central nervous system. It should prove useful for future studies of the functional role of SP in various regions of the central nervous system.

A. S. EISON
S. D. IVERSEN
Department of Experimental Psychology, University of Cambridge, Cambridge CB2 2QH, England
B. E. B. SANDBERG, S. P. WATSON
M. R. HANLEY, L. L. IVERSEN
MRC Neurochemical Pharmacology Unit, Medical Research Council Centre, Medical School, Hills Road, Cambridge CB2 2QH

References and Notes

1. M. Otsuka, S. Konishi, T. Takahashi, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 1922 (1975).
2. G. Zetler, *Adv. Biochem. Psychopharmacol.* **18**, 1 (1978).
3. R. A. Nicoll, C. Schenker, S. E. Leeman, *Annu. Rev. Neurosci.* **3**, 227 (1980).
4. P. C. Waldmeier, R. Kam, K. Stocklin, *Brain Res.* **159**, 223 (1978); H. Berger, K. Fechner, E. Albrecht, H. Niedrich, *Biochem. Pharmacol.* **28**, 3173 (1979).
5. C. M. Lee, B. E. B. Sandberg, M. R. Hanley, L. L. Iversen, *Eur. J. Biochem.* **114**, 315 (1981).
6. B. E. B. Sandberg *et al.*, *ibid.*, p. 329.
7. The relative potencies of DiMe-C7 (SP = 1.0) in various peripheral bioassays were as follows: contraction of guinea pig ileum (in presence of atropine), 0.05; enhancement of electrically driven contractions of rat vas deferens, 0.1; stimulation of salivation in rat, 0.02; and stimulation of plasma extravasation in rat forepaw, 0.1.
8. L. Stinus, A. E. Kelley, S. D. Iversen, *Nature (London)* **276**, 616 (1978).
9. A. E. Kelley, L. Stinus, S. D. Iversen, *Neurosci. Lett.* **11**, 335 (1979).
10. The [4-^{3}H-Phe^8]substance P (specific activity 23 Ci/mmole) was prepared and characterized as described (M. C. Allen, D. E. Brundish, R. Wade, B. E. B. Sandberg, M. R. Hanley, L. L. Iversen, *J. Med. Chem.*, in press). For microinfusion, it was diluted with standard SP to a final specific activity of 3.74 Ci/mmole. The (4-^{3}H-Phe^7)-DiMe-C7 (specific activity, 18.3 Ci/mmole) was prepared and characterized as described elsewhere (B. E. B. Sandberg, M. R. Hanley, S. P. Watson, D. E. Brundish, R. Wade, A. Eison, in preparation). For microinfusion, [^{3}H]DiMe-C7 was diluted with standard DiMe-C7 to a final specific activity of 2.95 Ci/mmole).
11. Rats under Equithesin anesthesia (3.75 ml/kg) were prepared with stereotaxically implanted 23-gauge stainless steel guide cannulas (12 mm) ending 3 mm above the VTA. Coordinates for the placement were, in millimeters, 3.5 posterior to bregma, ± 0.5 lateral to the midline, and 5.6 below skull [L. J. Pellegrino and A. J. Cushman, *A Stereotaxic Atlas of the Rat Brain* (Appleton-Century-Crofts, New York, 1967)]. Locomotor activity was recorded in cages (35 by 25 by 25 cm) fitted with a pair of photocells (130 mm apart and 10 mm above the cage floor). Interruptions of the beam were recorded on line with a Rockwell AIM 65 microprocessor. The rats were habituated to cages 2 hours daily for 3 days before testing and 2 hours on the test day. Data were recorded in 10-minute intervals for 90 minutes after infusion.
12. A. S. Eison, M. S. Eison, S. D. Iversen, *Brain Res.*, in press.
13. We thank R. Wade and D. Brundish, Ciba-Geigy, Horsham, England, for the labeled peptides. M.R.H. is a Helen Hay Whitney Foundation postdoctoral fellow and S.P.W. is a Medical Research Council scholar.

23 June 1981; revised 12 August 1981

Reduced Sympathetic Nervous System Responsivity Associated with the Relaxation Response

Abstract. *Sympathetic nervous system activity was assessed in experimental and control subjects who were exposed to graded orthostatic and isometric stress during monthly hospital visits. After the first session, the experimental subjects practiced a technique that elicited the relaxation response. Their concentrations of plasma norepinephrine during subsequent graded stresses were significantly higher. No such changes were noted in the control group. These results were then replicated in the control group in a crossover experiment. The groups did not differ in their heart rate and blood pressure responses. These observations are consistent with reduced norepinephrine end-organ responsivity after regular elicitation of the relaxation response.*

The relaxation response is defined by a set of integrated physiological changes that are elicited when a subject assumes a relaxed position in a quiet environment, closes his or her eyes, engages in a repetitive mental action, and passively ignores distracting thoughts (*1–4*). These behaviors are associated with physiological changes that include decreased oxygen consumption, heart rate (HR), arterial blood pressure (BP), respiratory rate, and arterial blood lactate (*4–6*). In addition, there are slight increases in skeletal muscle blood flow. All of these changes are different from those reported during sleep or quiet sitting (*4, 5, 7*). Although the physiological changes associated with the relaxation response are consistent with decreased sympathetic nervous system (SNS) activity, direct measurements of plasma norepinephrine (NE) levels have been reported to be either unchanged (*8*) or variable (*9*). In the current prospective, crossover investigation, we measured SNS reactivity in experimental subjects before and after they regularly elicited the response and compared the results with those of control subjects.

Medical histories were taken and physical examinations performed on 32 subjects. Two were excluded because of chronic illness. Informed consent was obtained from the remaining 30 subjects, who were randomly assigned to either an experimental or a control group. Nineteen subjects completed the entire investigation with ten in the experimental group (seven males, three females) and nine in the control group (four males, five females). The average age of the experimental group was 25.2 ± 3.5 years and of the control group, 25.5 ± 3.2 years.

The subjects were admitted to the Clinical Research Center of the Beth Israel Hospital in the early evening so that a 10- to 12-hour adaptation period preceded testing the next morning. During this adaptation period, maximum handgrip-tension levels were established for each subject. Each subject was awakened approximately 30 minutes before testing and had a 21-gauge intravenous catheter inserted in the antecubital vein of the nondominant arm. Sequential blood samples (10 ml each) were drawn for plasma NE analyses at five different times (−10, 0, +5, +10, and +15 minutes). When the first two samples (−10 and 0) were taken, the subjects were supine; the subjects then stood up. After 5 minutes of standing, the +5 sample was drawn. For the next 5 minutes, the subjects remained standing and also gripped at a tension corresponding to 30 percent of maximum. At the end of this 5-minute period, the +10 sample was drawn. In the subsequent 5 minutes, subjects remained standing and gripped at 100 percent of maximum grip tension. The +15 sample was then drawn. Immediately prior to each blood drawing, HR was measured by palpation of the radial pulse and BP by auscultation.

Blood samples were kept on ice

0036-8075/82/0108-0190$01.00/0