for at least twice the control value. The substances were dissolved in 20 µl saline and injected directly into the right lateral horn of the third ventricle according to CHERMAT and SIMON[15]. The doses of the compounds administered ICV were expressed in mole/animal.

Results. The time-response curves showing the analgesic effect of morphine and LPH-(61–91) are of similar character (Figure 1). The analgesic effect developed gradually, reached the maximum between 30 and 60 min and was sustained for hours. The slow onset of morphine's action when administered intracerebrally was also shown by others[16,17]. The effect of both morphine and LPH-(61–91) could be antagonized by naloxone, given subcutaneously 30 min prior to ICV administration of the compounds (Figure 1). The reaction times in the morphine or LPH-(61–91) + naloxone treated group were significantly lower ($p < 0.05$) than in the animals given morphine or LPH-(61–91) alone, at 15, 30, 45 and 60 min and 5, 15, 30 and 45 min, respectively. As the Figure 2 shows, the dose-response curves plotted from the data transformed for probit analysis[18] are parallel. The ED_{50} values were 7.7×10^{-9} (5.4–11.0) mole/animal for morphine and 4×10^{-10} (2.7–5.9) mole/animal for LPH-(61–91), i.e. the potency ratio was as high as 19.3 (11.3–32.7) calculated on molar basis. The Met-enkephalin and LPH-(61–79) also showed weak analgesic action (the latter being the more efficient) but their activity was too low for calculating the ED_{50} values tested at doses as high as 6.7×10^{-7} and 8×10^{-8} mole/animal, respectively.

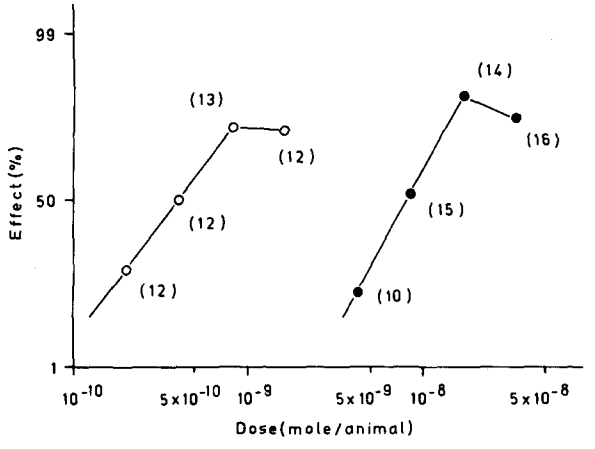

Fig. 2. Dose-response curves of the analgesic effect of morphine (●) and LPH-(61–91) (○), plotted according to LITCHFIELD and WILCOXON[18].

Discussion. According to the data in the literature, a β-LPH fragment, to exhibit opiate agonist properties in vitro, must contain residues 61–65 at the *N*-terminus. However, this segment is not sufficient to produce remarkable in vivo efficacy. For the latter, the whole C-terminal fragment of β-LPH is required. It may be speculated that LPH-(61–91) contains further binding site(s) within residues 66–91 and/or this part of the molecule has a protective role against the inactivating enzyme(s) of the brain. In any case, it seems probable that the LPH-(61–91) is a (the ?) physiological modulator in mammalian brain in supression of the pain reaction.

[15] R. CHERMAT and P. SIMON, J. Pharmac. Paris *6*, 489 (1975).
[16] A. PERT and T. YAKS, Brain Res. *80*, 135 (1974).
[17] F. BERGMAN, M. CHAIMOVITZ, V. PASTERNAK and A. RAMU, Br. J. Pharmac. *51*, 197 (1974).
[18] J. T. LITCHFIELD, Jr, and F. WILCOXON, J. Pharm. exp. Ther. *96*, 99 (1949).

7-Chloro-3-(4-methyl-1-piperazinyl)-4H-1,2,4-benzothiadiazine-1,1-dioxide, a new antihypertensive agent

M. Shimizu, K. Yoshida, T. Kadokawa, N. Hatano, J. Kuwashima, K. Nakatsuji, I. Nose and M. Kobayashi

Department of Pharmacology, Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Enoki 33–94, Suita, Osaka, 564 (Japan), 30 July 1976

Summary. 7-Chloro-3-(4-methyl-1-piperazinyl)-4H-1,2,4-benzothiadiazine-1,1-dioxide (DU-717) is a new compound having sustained antihypertensive activity in a similar manner to that of hydrochlorothiazide. However, this compound shows neither diuretic nor hyperglycemic effect, being different from those of hydrochlorothiazide or diazoxide.

During the process of pharmacological studies on a series of 1,2,4-benzothiadiazine-1,1-dioxide derivatives, we have found that some compounds show an antihypertensive activity without diuretic and hyperglycemic effect in experimental animals. 7-Chloro-3-(4-methyl-1-piperazinyl)-4H-1,2,4-benzothiadiazine-1,1-dioxide (DU-717) is one of such compounds with the chemical structure shown in figure 1.

Hypotensive activity was investigated in male spontaneously hypertensive rats (SHR)[1], 12–16 weeks of age and 270–320 g of body weight, with systolic blood pressure levels above 170 mm Hg, or in normotensive Wistar rats (NWR), 300–350 g of body weight, with systolic blood pressure levels 120–140 mm Hg. DU-717, diazoxide and hydrochlorothiazide (HCT) were suspended in 0.5% tragacanth solution and administered orally by a stomach tube. Blood pressure was measured with the tail-plethysmographic method after warming the animal in a heated box maintained at 38 °C for 10 min without anesthesia. Hypotensive activity of a single administration was investigated in SHR and blood pressure was measured prior to dosing and 1, 3, 5, 7 and 24 h after an oral administration. DU-717 and HCT at a dose of 1000 mg/kg showed no effect on blood pressure. However, diazoxide at a dose of 100 mg/kg significantly lowered blood pressure and the maximum effect was observed 5 h after an oral administration. Moreover, hypotensive activity of a repeated administration was investigated in SHR and NWR. DU-717, diazoxide and HCT were administered orally to SHR and NWR once a day for 10 successive days. Blood pressure was measured prior to dosing and 5 h after an oral administration on alternate days. At a dose of 1 mg/kg/day, DU-717, diazoxide and HCT showed

1 K. Okamoto and K. Aoki, Jap. Circul. J. *27*, 282 (1963).

no effect on blood pressure in SHR throughout the treatment period. However, at doses larger than 3 mg/kg/day, each drug lowered blood pressure. As shown in figure 2, a repeated dose (10 mg/kg/day) of DU-717 caused a sustained and significant reduction in blood pressure at 5–7th day after the first administration without marked daily fluctuations. Following cessation of medication, the lowered blood pressure slowly returned to pretreatment levels within 5 days. Diazoxide and HCT produced results similar to those obtained with DU-717. Moreover, in NWR diazoxide significantly lowered blood pressure at a single dose of 300 mg/kg, but DU-717 and HCT began to cause hypotensive effect after 5–7 days at a repeated dose of 300 mg/kg/day. These results obtained indicate that the hypotensive activity of DU-717 was mild, but sustained in a similar manner to that of HCT.

Effects of DU-717, diazoxide and HCT on urine volume and electrolyte excretion were studied in SHR and NWR. Animals received orally each drug in 25 ml/kg of 0.9% saline. Following the treatment, the animals were placed in metabolic cages for a 5-h-urine collection. Total amount of sodium and potassium was determined flame photometrically by the Technicon Auto-analyzer. Diazoxide remarkably decreased urine volume and electrolyte excretion at the doses larger than 30 mg/kg in SHR and NWR. These effects were dose-dependent. In contrast with the oliguric effect of diazoxide, HCT caused a significant increase in urine volume and electrolyte excretion at the oral doses larger than 0.1 mg/kg in SHR and NWR. However, DU-717 caused no significant effect on urine volume and electrolyte excretion in SHR and NWR, even at the high doses of 100 and 300 mg/kg, respectively. Furthermore, a crossover study was conducted in 5 male Beagle dogs, weighing 13–17 kg, to investigate the effect of DU-717, diazoxide and HCT on the urine volume and electrolyte excretion. Urine was continuously collected by an urethral catheter at 1–2-h-intervals after an oral administration. After a week of rest, the dogs were retested on a crossover basis. Diazoxide significantly decreased urine volume and electrolyte excretion at a dose of 30 mg/kg. HCT caused a distinct increase in urine volume and electrolyte excretion at a dose of 1 mg/kg. However, DU-717 caused no appreciable effect on urine volume and electrolyte excretion at a higher dose of 100 mg/kg. These results indicate that DU-717 shows neither diuretic nor oliguric activity.

Carbohydrate metabolism was investigated in NWR, SHR and alloxan-diabetic rats by an oral or intraperitoneal administration of DU-717, diazoxide and HCT. Blood was drawn from the retro-orbital venous plexus of the rats and blood glucose levels were determined by an enzymatic method[2]. Moreover, the rats receiving orally each drug once a day for 14–40 successive days were tested for tolerance to glucose. The single or repeated oral administration (300 mg/kg) of DU-717 showed no effect on blood glucose levels and glucose tolerance test (GTT) in NWR, SHR and alloxan-diabetic rats. In addition, at a single intraperitoneal dose of 300 mg/kg, DU-717 did not affect blood glucose, liver glycogen, plasma insulin and plasma corticosterone levels in NWR. However, the oral administration of diazoxide resulted in a marked rise in blood glucose levels but HCT caused no effect in NWR. The intraperitoneal administration of diazoxide and HCT caused a remarkable increase in blood glucose and plasma corticosterone levels with a simultaneous decrease in liver glycogen level in NWR. Moreover, the repeated oral administration of diazoxide and HCT caused abnormality of GTT in SHR, and HCT enhanced abnormality of GTT in alloxan-diabetic rats. Consequently, as shown in the table, the above findings

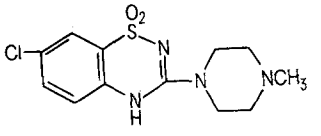

Fig. 1. Chemical structure of DU-717.

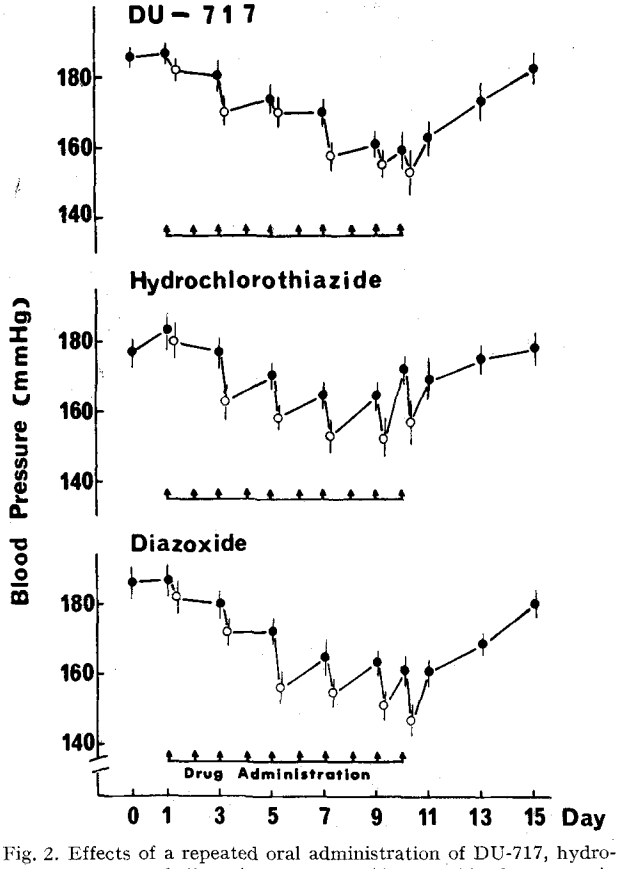

Fig. 2. Effects of a repeated oral administration of DU-717, hydrochlorothiazide and diazoxide (10 mg/kg/day) on blood pressure in conscious SHR. ●, Blood pressure obtained before an oral administration; ○, blood pressure obtained 5 h after an oral administration. Each point is the mean of individual groups and the vertical lines represent the standard error of the mean.

2 O. H. Lowry, J. V. Passonneau, F. X. Hasselberger and D. W. Schulz, J. biol. Chem. *239*, 18 (1964).

Summary of comparative effects of DU-717, hydrochlorothiazide and diazoxide

Compound	Anti-hypertensive effect	Diuretic effect	Oliguric effect	Carbohydrate metabolism
DU-717	+	–	–	–
Hydro-chlorothiazide	+	+	–	+
Diazoxide	+	–	+	+

+, Significant effect; –, no change.

clearly indicate that DU-717 shows a dose-related and sustained reduction in blood pressure without any change in urine volume, electrolyte excretion and carbohydrate metabolism. Subacute toxicity of DU-717 was examined in male Sprague-Dawley rats. At a dose of 1000 mg/kg p.o. for 30 days, DU-717 caused no significant change in the weight gain and organ weight or no appreciable effect on the hematobiochemical findings as compared with the control. The general hemodynamic actions of DU-717, and the mechanisms responsible for its hypotensive effect, are currently under investigation.

It may be concluded that DU-717 is a compound having a novel profile, different from those of the antihypertensive drugs of benzothiadiazine diuretics or diazoxide in its pharmacological properties and underlying mode of action. It seems, therefore, worthwhile to study the clinical efficacy of this compound in the treatment of patients with essential hypertension.

Effects of 1-alkyl-1, 2, 3, 4-tetrahydrocarbazole-1-ethanamines and related compounds, potential antidepressants, on biogenic amine uptake mechanisms

T. A. Pugsley and W. Lippmann[1]

Biochemical Pharmacology Department, Ayerst Research Laboratories, Box 6115, Montreal (Quebec, Canada H3C 3J1), 30 April 1976

Summary. A new series of compounds structurally related to the potential antidepressant tandamine, i.e., the 1-alkyl-1,2,3,4-tetrahydrocarbazole-1-ethanamines, inhibit the noradrenaline uptake mechanism and are relatively ineffective in inhibiting the serotonin uptake mechanism in vivo. The potency of the most effective compound (9-ethyl-N,N,1-trimethyl-1,2,3,4-tetrahydrocarbazole) is similar to that of desimipramine and is of potential use in the treatment of endogenously depressed patients.

Recent studies[2–5] demonstrate that a new potential antidepressant drug tandamine hydrochloride (9-ethyl-*N, N,* 1-trimethyl-1, 2, 3, 4-tetrahydrothiopyrano [3, 4-b] indole-1-ethanamine hydrochloride) (Table 1) possesses both a biochemical and pharmacological profile in animals qualitatively similar to, but lacking the central anticholinergic activity of, the tricyclic antidepressant desimipramine (DMI). Tandamine is greater, or equivalent, in potency to DMI as an inhibitor of noradrenaline (NA) reuptake mechanism and, like DMI, is relatively ineffective in inhibiting the 5-HT uptake mechanism[2–4]. In the present studies a series of compounds, structurally related to tandamine (Table 1), i.e., the 1-alkyl-1, 2, 3, 4-tetrahydrocarbazole-1-ethanamines and related compounds (cycloalkanoindoles), have been examined for their inhibitory activities on NA and 5-HT reuptake mechanisms; there is considerable evidence that the antidepressive action of certain known tricyclic antidepressants may be due to their abilities to cause inhibition of the neuronal reuptake of NA[6], 5-HT[7], or both.

Materials and methods. The cycloalkanoindoles were synthesized by Drs A. Asselin and L. G. Humber and Mrs J. Komlossy, Ayerst Research Laboratories[8]. Desimipramine hydrochloride (Pertofrane) and imipramine hydrochloride (Tofranil) (I) were gifts from Ciba-Geigy.

The determination of the effects of the compounds on ^{3}H-NA uptake and release in the hearts of albino mice (23–25 g, Canadian Breeding Laboratories) was carried out as previously described[3, 9] as was their ability to potentiate the 5-hydroxytryptophan (5-HTP)-induced behavioural syndrome in mice[7]. The determination of the effects of the test compound on the α-4-dimethyl-3-hydroxy-phenylethylamine (H77/77)-induced depletion of brain NA and dopamine (DA) was carried out[11] and the concentrations of brain NA and DA were determined[12–14]. Percent inhibition exhibited by the test compounds of the H77/77-induced decline in brain NA was calculated by utilizing the formula employed by Bruinvels[15].

Results and discussion. Compounds *1, 2, 3, 5, 6, 8, 9* and *10,* and also DMI and I, when given before the ^{3}H-NA, decreased the ^{3}H-NA concentrations in mouse heart displaying ED_{50}'s < 10 mg/kg, i.p. (Table 1). None of these compounds when administered at 10 mg/kg, i.p., after the ^{3}H-NA altered the ^{3}H-NA as compared to controls [e.g., controls: 48,630 ± 1784; *2*: 53,137 ± 1167; I: 53,810 ± 2338 (cpm/g ± S.E.)]. Thus, the cycloalkanoindoles, like DMI and I, blocked the uptake and did not cause an increased release of ^{3}H-NA. It is apparent that the presence of an ethyl group on the indole nitrogen, a methyl group and a dimethylaminoethyl side chain at position 1 in the 1-alkyl-1, 2, 3, 4-tetrahydrocarbazole-1-ethanamines, i.e., compound *2*, results in maximal activity (ED_{50}: 0.7 mg/kg, i.p.). Lengthening the chain on the ring nitrogen to the propyl (*5*) or shortening the length to a methyl (*1*) decreased the activity. Reduced activity was also caused by replacement of the dimethylamino group of the side chain by a methylamino (*3*). The presence of an ethyl group at position 8 (*7*) caused a decrease in activity and a chloro group at position 6 (*8*) also re-

[1] The authors acknowledge the technical assistance of Mrs J. Farnsworth, Mrs B. Gut and Mr J. Lacasse.

[2] W. Lippmann and T. A. Pugsley, Pharmacologist *17*, 258 (1975).
[3] W. Lippmann and T. A. Pugsley, Biochem. Pharmac. *25*, 1179 (1976).
[4] T. A. Pugsley and W. Lippmann, Psychopharmacologia *47*, 33 (1976).
[5] I. Jirkovsky, L. G. Humber, K. Voith, M.-P. Charest, T. A. Pugsley and W. Lippmann, 169th ACS National Meeting, Philadelphia, Pa., April 1975, Abstr. Med. 34.
[6] J. Glowinski and J. Axelrod, Pharmac. Rev. *18*, 775 (1966).
[7] A. Carlsson, J. Jonason, M. Lindqvist and K. Fuxe, Brain Res. *12*, 456 (1969).
[8] A. A. Asselin, L. G. Humber, J. Komlossy and M.-P. Charest, J. Med. Chem. (in press).
[9] W. Lippmann, J. Med. *2*, 250 (1971).
[10] A. Carlsson, H. Corrodi, K. Fuxe and T. Hökfelt, Eur. J. Pharmac. *5*, 357 (1969).
[11] A. Carlsson, H. Corrodi, K. Fuxe and T. Hökfelt, Eur. J. Pharmac. *5*, 367 (1969).
[12] L. G. Whitby, J. Axelrod and H. Weil-Malherbe, J. Pharmac. exp. Ther. *132*, 193 (1961).
[13] U. S. Von Euler and I. Floding, Acta Physiol. scand. *33*, Suppl. *118*, 45–47 (1955).
[14] R. Laverty and K. M. Taylor, Anal. Biochem. *22*, 269 (1968).
[15] J. Bruinvels, Br. J. Pharmac. *42*, 281 (1971).