A TRH ANALOG (DN-1417): EFFECTS ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN CONSCIOUS AND PENTOBARBITALIZED RATS DETERMINED BY THE AUTORADIOGRAPHIC 2-DEOXY- $[^{14}\text{C}]$ GLUCOSE METHOD

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Abstract—Effects of a TRH (thyrotropin-releasing hormone) analog, DN-1417 (γ -butyrolactone- γ -carbonyl-L-histidyl-L-prolinamide citrate), with regard to the rate of local cerebral glucose utilization (LCGU) were assessed using the autoradiographic 2-deoxy-D- $[^{14}\text{C}]$ glucose method to clarify the possible sites of action in conscious and pentobarbitalized rats. DN-1417 (0.5–5 mg/kg, i.v.) significantly shortened the pentobarbital-induced sleeping time in a dose dependent manner, and this effect of DN-1417 was reduced by intracerebroventricular (i.c.v.) pretreatment with atropine methylbromide (20 μg). DN-1417 (5 mg/kg, i.v.) by itself slightly increased the LCGU in the thalamus dorsomedial nucleus and mammillary body and significantly increased it in the septal nucleus. Pentobarbital (30 mg/kg, i.v.) alone produced a marked and diffuse reduction in LCGU throughout the brain at a rate of about 46% of the control. DN-1417 (5 mg/kg, i.v.) markedly reversed the reduction of LCGU induced by pentobarbital. The antagonistic effect of DN-1417 in LCGU was about twice as potent as that of TRH. Locally, significant reversal effects by DN-1417 were observed in the hypothalamus, septal nucleus, hippocampus, mammillary body, thalamus dorsomedial nucleus, caudate-putamen, nucleus accumbens, pontine gray matter and so on. These actions of DN-1417 were almost completely abolished in all areas except for the visual cortex under the condition of pretreatment with atropine methylbromide (20 μg , i.c.v.). On the other hand, pimozide (1 mg/kg, i.p.) augmented the actions of DN-1417. Thus, DN-1417 seems to act on the level of consciousness via a reticulo-thalamo-cortical system, and reverses the LCGU reduction induced by pentobarbital via a cholinergic mechanism. The hypothalamus appears to be a crucial site in mediation of the antagonistic action of DN-1417 on pentobarbital.

Following observations of the clinical (1–5) and experimental effects (6–10) of TRH, we have looked for a more potent and

long-acting analog that does not have hormonal action. TRH is short-acting in the central nervous system (CNS) due to its short half-life in tissues (11, 12), and most of its effects in the CNS are independent of the function of the hypothalamo-pituitary axis.

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Abbreviations used: $[^{14}\text{C}]$ DG, 2-Deoxy-D- $[^{14}\text{C}]$ glucose; ABTS, 2,2'-Azino-di(3-ethylbenzothiazoline)-6-sulphonate.

Among a number of TRH analogs, γ -butyrolactone- γ -carbonyl-L-histidyl-L-

prolinamide citrate (DN-1417) is the most potent with regard to the CNS activity, and it is without hormonal action (13). DN-1417 is approx. 2–6 times more potent and longer-acting than TRH: it increases motor activity, shortens pentobarbital- and ethanol-induced sleeping time, antagonizes pentobarbital- and reserpine-induced hypothermia, while its TSH-releasing activity is only approx. 1/40 that of TRH in rats (14).

Thus it seems of considerable importance, in evaluating the pharmacological mechanism and the possible action sites in brain, to study the effect of DN-1417 on local cerebral glucose utilization (LCGU) since there is a close relationship between energy metabolism and the level of functional activity in the CNS (15). We now report the antagonistic effects of DN-1417 against pentobarbital-induced reduction of LCGU in rats, and the results were compared with findings obtained with TRH.

Materials and Methods

Animals: Experiments were performed on male Sprague-Dawley (Jcl: SD, SPF) rats weighing 280–330 g for measurements of pentobarbital-induced sleeping time and rats weighing 350–450 g for measurements of LCGU and miscellaneous physiological parameters.

Materials: [^{14}C]DG (spec. act., 50–56 mCi/mmol) was purchased from New England Nuclear Corp. Other drugs, reagents and enzymes used were DN-1417 (γ -butyrolactone- γ -carbonyl-L-histidyl-L-prolinamide citrate, Takeda), TRH (L-pyroglutamyl-L-histidyl-L-prolinamide L-tartrate monohydrate, Takeda), pentobarbital Na (Mintal®, Tanabe), glucose-oxidase (Boehringer-Mannheim, grade II), peroxidase (Boehringer-Mannheim, grade II), ABTS® (Boehringer-Mannheim, Superior quality), atropine methylbromide (Tropin®, Takeda), and pimozone (Orap®, Fujisawa). Peptides

and pentobarbital were dissolved in 0.9% physiological saline (saline). The doses were expressed in terms of the weight of the salt.

Measurement of pentobarbital-induced sleeping time: DN-1417 (0.2–5 mg/kg) or saline was given to groups of 7–8 rats intravenously (i.v.) 10 min after the onset of loss of the righting reflex induced by intraperitoneal (i.p.) administration of pentobarbital (40 mg/kg). The time from the administration of DN-1417 to the time of regaining the righting reflex was taken as the sleeping time.

Atropine methylbromide (20 μg /rat) was given intracerebroventricularly (i.c.v.) as described previously (16), 5 min before and DN-1417 (5 mg/kg, i.v.) was administered 15 min after the injection of pentobarbital (30 mg/kg, i.v.). Percent shortening of the sleeping time by DN-1417 was calculated by the formula: $[1 - (\text{sleeping time in DN-1417-treated group} / \text{sleeping time in saline-treated control group})] \times 100$.

Measurement of rate of local cerebral glucose utilization: Surgical procedures, measurements of plasma glucose, plasma [^{14}C] concentration and brain tissue [^{14}C] concentration and calculation of LCGU were performed as described previously (16). An interval of 5 min between application of DN-1417 and infusion of [^{14}C]DG was taken as in the case of TRH (16). Pimozide (1 mg/kg, i.p.) or atropine methylbromide (20 μg , i.c.v.) was administered 4 hr and 5 min before, respectively; and DN-1417 (5 mg/kg, i.v.) or TRH (10 mg/kg, i.v.) was administered 15 min after administration of pentobarbital. Pentobarbital (30 mg/kg, i.v.) was administered 20 min before an intravenous infusion of 50 $\mu\text{Ci}/0.5$ ml of saline/rat of [^{14}C]DG over a 30 sec period.

Measurement of miscellaneous physiological parameters: Another series of experiments were performed to assess the effects of various drug treatments on physiological

parameters such as blood gases and general circulation. Groups of four rats were subjected to the same surgical procedures as for measurements of LCGU. Blood pressure, heart rate and arterial blood pH, $p\text{CO}_2$ and $p\text{O}_2$ were measured as described previously (16).

Statistics: Statistical comparison between different treatments was made by the Student's *t*-test.

Results

Antagonistic effect of DN-1417 on pentobarbital-induced sleep: As shown in Table 1, DN-1417 in doses of 0.5–5 mg/kg, i.v. significantly shortened the pentobarbital-induced sleeping time, in a dose dependent manner, when DN-1417 was injected 10 min after loss of the righting reflex by the adminis-

tration of pentobarbital (40 mg/kg, i.p.). Citric acid, a salt constituent of DN-1417, was not effective in a dose (1.7 mg/kg, i.v.) equivalent to the highest dose of DN-1417 (5 mg/kg, i.v.) used. The shortening action of DN-1417 (5 mg/kg) injected 15 min after the administration of pentobarbital (30 mg/kg, i.v.) was almost completely blocked by pretreatment with atropine methylbromide (20 μg , i.c.v.) (Table 2).

Effects of DN-1417 by itself and against pentobarbital in LCGU: The rates of LCGU in various brain structures of rats treated with DN-1417, pentobarbital alone and its combination with DN-1417 or TRH are given in Table 3. DN-1417 by itself slightly increased LCGU in the thalamus dorsomedial nucleus and mammillary body, as compared with the control; and a significant increase was found

Table 1. Effect of DN-1417 on pentobarbital-induced sleeping time in rats

Treatment	Dose (mg/kg, i.v.)	Sleeping time (mean $\pm$ S.E.M. min)	Shortening (%)
Saline	—	43.1 $\pm$ 1.2	—
Citric acid	1.7	45.7 $\pm$ 3.2	-6.0
DN-1417	0.2	39.9 $\pm$ 5.1	7.6
	0.5	34.8 $\pm$ 2.1	19.3 ^{a)}
	1.0	31.0 $\pm$ 2.6	28.3 ^{a)}
	5.0	25.4 $\pm$ 1.8	41.1 ^{a)}

All values are the mean $\pm$ S.E.M. of 7–8 rats. DN-1417, citric acid or saline was administered 10 min after loss of the righting reflex by the administration of pentobarbital (40 mg/kg, i.p.). Citric acid, a constituent of DN-1417, was not effective in a dose (1.7 mg/kg) equivalent to DN-1417 (5 mg/kg) used.

^{a)} Statistically significant level, $P<0.01$ vs. saline.

Table 2. Influence of atropine methylbromide (ATMB) on the antagonistic effect of DN-1417 against pentobarbital-induced sleeping time

Treatment	Dose	Sleeping time (mean $\pm$ S.E.M. min)	Shortening (%)
Saline	—	38.4 $\pm$ 2.1	—
DN-1417	5 mg/kg	18.4 $\pm$ 2.6 ^{a)}	52.1
ATMB	20 μg	45.4 $\pm$ 5.5	—
ATMB +DN-1417	20 μg		
	5 mg/kg	40.2 $\pm$ 3.2	11.5

All values are the mean $\pm$ S.E.M. of 5 rats. ATMB (20 μg , i.c.v.) was administered 5 min before and DN-1417 (5 mg/kg, i.v.) was administered 15 min after the injection of pentobarbital (30 mg/kg, i.v.).

^{a)} Statistically significant level, $P<0.001$ vs. saline.

Table 3. Effects of DN-1417, pentobarbital alone and its combination with DN-1417 or TRH on local cerebral glucose utilization in rats

Structure	Local cerebral glucose utilization ($\mu\text{mol}/100\text{ g}/\text{min}$)				
	Control	DN-1417	Pentobarbital		
			Saline	DN-1417	TRH
Visual cortex	71.7 $\pm$ 4.6	87.5 $\pm$ 11.3	31.8 $\pm$ 4.0 ^{e)}	46.0 $\pm$ 3.9 ^{d)}	48.7 $\pm$ 5.7 ^{d)}
Auditory cortex	102.0 $\pm$ 7.3	112.3 $\pm$ 2.9	50.1 $\pm$ 6.4 ^{e)}	61.8 $\pm$ 3.7	64.6 $\pm$ 7.1
Parietal cortex	80.6 $\pm$ 5.4	88.6 $\pm$ 6.6	33.7 $\pm$ 5.5 ^{e)}	46.9 $\pm$ 4.2	51.2 $\pm$ 5.2 ^{d)}
Sensory-motor cortex	84.3 $\pm$ 5.4	91.3 $\pm$ 4.6	36.1 $\pm$ 4.1 ^{e)}	53.2 $\pm$ 4.5 ^{d)}	48.6 $\pm$ 5.1
Olfactory cortex	65.6 $\pm$ 5.0	69.7 $\pm$ 7.2	22.9 $\pm$ 8.2 ^{b)}	43.8 $\pm$ 3.5 ^{d)}	50.2 $\pm$ 5.4 ^{d)}
Frontal cortex	67.8 $\pm$ 5.5	71.2 $\pm$ 6.6	26.5 $\pm$ 5.3 ^{e)}	38.3 $\pm$ 4.1	43.0 $\pm$ 5.1
Thalamus: Lateral nucleus	84.3 $\pm$ 4.9	93.8 $\pm$ 8.4	30.2 $\pm$ 5.8 ^{e)}	48.9 $\pm$ 4.1	46.5 $\pm$ 5.3
Ventral nucleus	69.7 $\pm$ 5.1	85.5 $\pm$ 6.2	29.2 $\pm$ 6.4 ^{b)}	44.7 $\pm$ 3.6	44.3 $\pm$ 6.0
Dorsomedial nucleus	103.0 $\pm$ 6.7	125.0 $\pm$ 10.2	42.8 $\pm$ 6.5 ^{e)}	67.0 $\pm$ 4.3 ^{d)}	65.3 $\pm$ 7.8
Habenula	95.4 $\pm$ 5.8	98.4 $\pm$ 6.2	45.2 $\pm$ 6.5 ^{e)}	59.6 $\pm$ 3.9	54.6 $\pm$ 6.4
Subthalamic nucleus	67.4 $\pm$ 5.4	66.4 $\pm$ 4.2	33.0 $\pm$ 2.9 ^{e)}	40.2 $\pm$ 3.2	35.3 $\pm$ 4.1
Medial geniculate body	74.9 $\pm$ 5.9	75.6 $\pm$ 5.4	29.5 $\pm$ 8.4 ^{b)}	40.4 $\pm$ 3.4	40.8 $\pm$ 5.8
Lateral geniculate body	65.4 $\pm$ 4.6	71.0 $\pm$ 3.5	28.1 $\pm$ 4.4 ^{e)}	41.2 $\pm$ 2.6 ^{d)}	42.2 $\pm$ 4.8
Hypothalamus	44.6 $\pm$ 4.1	49.2 $\pm$ 2.8	19.1 $\pm$ 1.6 ^{e)}	36.6 $\pm$ 2.6 ^{f)}	31.6 $\pm$ 3.4 ^{d)}
Mammillary body	105.6 $\pm$ 8.3	124.9 $\pm$ 7.6	41.4 $\pm$ 4.7 ^{e)}	77.0 $\pm$ 9.7 ^{d)}	50.7 $\pm$ 3.7
Hippocampus: Ammon's horn	62.4 $\pm$ 5.9	67.8 $\pm$ 3.3	34.9 $\pm$ 5.0 ^{b)}	50.1 $\pm$ 3.0 ^{d)}	46.3 $\pm$ 4.9
Dentate gyrus	43.8 $\pm$ 5.6	46.7 $\pm$ 3.8	22.0 $\pm$ 3.5 ^{a)}	34.8 $\pm$ 4.0 ^{d)}	34.3 $\pm$ 3.4 ^{d)}
Amygdala	43.2 $\pm$ 5.0	44.4 $\pm$ 3.1	27.8 $\pm$ 8.1	35.1 $\pm$ 3.2	33.7 $\pm$ 5.7
Septal nucleus	38.8 $\pm$ 2.2	49.5 $\pm$ 3.8 ^{a)}	14.5 $\pm$ 3.7 ^{e)}	32.2 $\pm$ 2.0 ^{e)}	32.1 $\pm$ 4.0 ^{d)}
Caudate-putamen	85.7 $\pm$ 5.7	92.4 $\pm$ 4.9	31.4 $\pm$ 5.4 ^{e)}	49.4 $\pm$ 2.2 ^{d)}	53.6 $\pm$ 6.4 ^{d)}
Nucleus accumbens	64.5 $\pm$ 7.2	73.5 $\pm$ 5.8	27.7 $\pm$ 6.6 ^{b)}	50.0 $\pm$ 3.5 ^{d)}	52.4 $\pm$ 4.4 ^{d)}
Globus pallidus	32.3 $\pm$ 3.3	39.2 $\pm$ 5.8	12.2 $\pm$ 1.4 ^{e)}	29.2 $\pm$ 6.3 ^{d)}	24.5 $\pm$ 3.5 ^{d)}
Substantia nigra	61.3 $\pm$ 7.8	53.5 $\pm$ 6.1	17.5 $\pm$ 4.1 ^{b)}	32.8 $\pm$ 2.6 ^{d)}	30.6 $\pm$ 3.3 ^{d)}
Raphe nucleus	71.1 $\pm$ 3.7	71.9 $\pm$ 5.6	24.6 $\pm$ 5.0 ^{e)}	39.0 $\pm$ 4.5	40.9 $\pm$ 4.7 ^{d)}
Locus caeruleus	85.8 $\pm$ 3.9	94.6 $\pm$ 2.8	31.8 $\pm$ 4.7 ^{e)}	46.2 $\pm$ 4.9	46.1 $\pm$ 5.8
Vestibular nucleus	108.1 $\pm$ 6.3	109.2 $\pm$ 7.3	71.2 $\pm$ 6.5 ^{b)}	76.3 $\pm$ 6.3	81.8 $\pm$ 7.5
Cochlear nucleus	125.2 $\pm$ 7.4	123.8 $\pm$ 3.1	78.7 $\pm$ 13.6 ^{a)}	109.1 $\pm$ 9.2	113.6 $\pm$ 12.3
Superior olivary nucleus	136.2 $\pm$ 8.6	146.3 $\pm$ 8.7	90.5 $\pm$ 11.9 ^{a)}	119.0 $\pm$ 8.9	119.9 $\pm$ 16.8
Lateral lemniscus	103.8 $\pm$ 4.0	108.3 $\pm$ 4.0	71.9 $\pm$ 5.4 ^{b)}	74.8 $\pm$ 5.4	93.3 $\pm$ 8.1
Inferior colliculus	156.7 $\pm$ 9.2	163.9 $\pm$ 3.7	81.6 $\pm$ 10.9 ^{e)}	99.6 $\pm$ 3.6	110.0 $\pm$ 10.3
Superior colliculus	65.4 $\pm$ 5.8	70.3 $\pm$ 5.7	30.7 $\pm$ 5.3 ^{b)}	51.7 $\pm$ 3.9 ^{d)}	46.6 $\pm$ 6.9
Pontine gray matter	45.6 $\pm$ 2.9	49.7 $\pm$ 3.3	19.8 $\pm$ 3.2 ^{e)}	33.7 $\pm$ 3.8 ^{d)}	32.1 $\pm$ 3.5
Cerebellar cortex	44.2 $\pm$ 6.7	42.7 $\pm$ 3.8	17.9 $\pm$ 3.8 ^{b)}	27.4 $\pm$ 3.5	31.7 $\pm$ 4.4 ^{d)}
Cerebellar nucleus	78.7 $\pm$ 4.6	80.8 $\pm$ 7.3	45.9 $\pm$ 5.2 ^{b)}	50.6 $\pm$ 5.2	53.9 $\pm$ 5.0

All values are the mean $\pm$ S.E.M. of 5 rats. DN-1417 (5 mg/kg, i.v.) was administered 5 min before the infusion of [^{14}C]DG. Pentobarbital (30 mg/kg, i.v.) was injected 15 min before the injection of DN-1417 (5 mg/kg, i.v.), TRH (10 mg/kg, i.v.) or saline, which was administered 5 min before the infusion of [^{14}C]DG. Statistically significant level: ^{a)} $P<0.05$, ^{b)} $P<0.01$, ^{c)} $P<0.001$ vs. control and ^{d)} $P<0.05$, ^{e)} $P<0.01$, ^{f)} $P<0.001$ vs. pentobarbital alone.

in the septal nucleus ($P<0.05$). Figure 1 shows the representative autoradiographs of the coronal section at the level of the septal nucleus. The dark areas in the autoradiograph represent high glucose utilization.

Throughout the brain except for the amygdala, pentobarbital induced a marked and diffuse reduction in LCGU to about 46% of the control. DN-1417 and TRH given 15 min after pentobarbital significantly

Fig. 1. Representative autoradiographs of coronal sections at level of the septal nucleus in rats. Autoradiographs were taken from a control rat (A) and a rat treated with DN-1417 (5 mg/kg, i.v.) (B). Abbreviation used: LS, septal nucleus.

reversed the reduction in LCGU induced by pentobarbital. The effect of DN-1417 (5 mg/kg) was roughly equipotent to TRH (10 mg/kg). The structures reversed by DN-1417 did not always coincide with those by TRH and were observed in the following 16 of 34 structures examined: cerebral cortex (visual, sensory-motor and olfactory cortices), diencephalon (thalamus dorsomedial nucleus, hypothalamus, mammillary body and lateral geniculate body), limbic areas (hippocampus ammon's horn and dentate gyrus and septal nucleus), basal ganglia (caudate-putamen, nucleus accumbens and globus pallidus) and mesencephalon (substantia nigra, superior colliculus and pontine gray matter).

Influence of pimozide or atropine methylbromide on the antagonistic effect of DN-

1417 against pentobarbital in LCGU: A second series of experiments were performed with pimozide and atropine methylbromide. The antagonistic effect of DN-1417 on pentobarbital in LCGU was augmented by a dopamine receptor blocker, pimozide (1 mg/kg, i.p.), but a cholinergic blocker, atropine methylbromide (20 μ g, i.c.v.), abolished the effect of DN-1417 in all structures except for the visual cortex (Fig. 2). Figure 3 shows representative autoradiographs of coronal sections at the level of the hypothalamus under conditions of various treatments. In the hypothalamus, DN-1417 antagonized the effect of pentobarbital, and it was blocked by atropine methylbromide.

Effects of various drug treatments on miscellaneous physiological parameters: Physiological parameters were measured before and at two points of time after saline administration instead of [14 C]DG, which corresponded to 10 and 45 min after the beginning of the [14 C]DG infusion in the experiment of LCGU, respectively (Table 4). Pentobarbital dramatically reduced blood pressure to about 50–60 mmHg and heart rate to about 60–70% of the control immediately after the injection. The recovery was gradual, and several minutes after the injection, the systolic blood pressure returned to about 90–100 mmHg. This recovery was accelerated by the administration of either DN-1417 or TRH. The heart rate did not recover so rapidly and was little influenced by the administration of DN-1417 or TRH. The depression of respiration induced by pentobarbital tended to raise the blood pCO₂ and lower the blood pO₂. As a result, the blood pH shifted slightly to acidic side from pH 7.4.

Discussion

In a foregoing paper (16), we reported that TRH clearly shortened the pentobarbital-induced sleeping time and the effect of TRH was almost completely blocked by pretreat-

Fig. 2. Influence of pimoziide (PMZ) or atropine methylbromide (ATMB) on the antagonistic effect of DN-1417 against pentobarbital in local cerebral glucose utilization in rats. All values are the mean of 5 animals. DN-1417 (5 mg/kg, i.v.) was administered 15 min after the injection of pentobarbital. (A) Influence of PMZ on the antagonistic effect of DN-1417 against pentobarbital. PMZ (1 mg/kg, i.p.) was administered 4 hr before the injection of pentobarbital (30 mg/kg, i.v.). Statistically significant level: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, pentobarbital+PMZ (shaded bar) vs. pentobarbital+PMZ+DN-1417 (white bar). (B) Influence of ATMB on the antagonistic effect of DN-1417 against pentobarbital. ATMB (20 μg , i.c.v.) was administered 5 min before the injection of pentobarbital (30 mg/kg, i.v.). Statistically significant level: * $P < 0.05$, pentobarbital+ATMB (dotted bar) vs. pentobarbital+ATMB+DN-1417 (white bar).

ment with atropine methylbromide (20 μg , i.c.v.) in rats. TRH by itself (5 mg/kg, i.v.) reduced LCGU in the whole brain to about 80% of the control, and yet it reversed the reduction of LCGU induced by pentobarbital (30 mg/kg, i.v.). The effect of TRH was abolished by pretreatment with atropine methylbromide (20 μg , i.c.v.).

A TRH analog, DN-1417, shortened the pentobarbital-induced sleeping time and

antagonized the reduction of LCGU induced by pentobarbital to a greater extent than did TRH. These effects were also abolished by atropine methylbromide (20 μg , i.c.v.). Unlike TRH, DN-1417 by itself slightly increased LCGU in the thalamus dorsomedial nucleus and mamillary body; and especially, a significant increase was noted in the septal nucleus. TRH and DN-1417 have different actions on LCGU, suggesting that DN-1417

Fig. 3. Representative autoradiographs of coronal sections at the level of the hypothalamus in rats. Autoradiographs were taken from a control rat and rats treated with DN-1417, pentobarbital, pentobarbital + DN-1417, pentobarbital-atropine methylbromide (ATMB)+DN-1417, and pentobarbital+pimozide (PMZ)+DN-1417. PMZ (1 mg/kg, i.p.) was administered 4 hr before and ATMB (20 μ g, i.c.v.) was administered 5 min before the injection of pentobarbital (30 mg/kg, i.v.). The rat was treated with DN-1417 (5 mg/kg, i.v.) at 15 min after the injection of pentobarbital.

may act on the CNS in a manner different from TRH.

The mammillary body in the rat receives input from the ventromedial hypothalamus (17) and sends efferents into the mammillo-thalamic tract which terminates in the cell groups within the anterior thalamic nuclei (18). It also connects with the frontal cortex via the anterior thalamus (19). From the autoradiographs, the thalamus dorsomedial nucleus of the present studies corresponds to a part of the anterior thalamic nuclei. The reticulo-thalamo-cortical system that includes the above regions plays a significant role in attention, consciousness, sleep and wakefulness (20–22). Therefore, the increase in LCGU in the mammillary body, thalamus dorsomedial nucleus and some parts of the cerebral cortex suggests that DN-1417 may stimulate a reticulo-thalamo-cortical system

and control the level of consciousness. These effects of DN-1417 were more evident under the condition of pentobarbital-induced sleep. DN-1417 did not produce any change in LCGU in the nucleus accumbens, in which TRH stimulated [3 H]dopamine release was observed in the *in vitro* experiments (23, 24). The nucleus accumbens has been suggested as a site of motor stimulant action by TRH and DN-1417 in rats (9).

Pentobarbital induced a marked and diffuse reduction in LCGU in almost all the cerebral structures, as shown in Table 3. DN-1417 significantly reversed the effect of pentobarbital in the various structures, and the effect of DN-1417 was roughly twice as potent as that of TRH in the rat whole brain. The most sensitively reversed structures were the hypothalamus and septal nucleus, followed by the hippocampus, mammillary

Table 4. Effects of various treatments on miscellaneous physiological parameters in rats

Treatment	Time (min)	pH	pCO ₂ (mm Hg)	pO ₂ (mm Hg)	Heart rate (beat/min)	Blood pressure (mm Hg)
Saline	Control	7.39±0.01	35.4±2.7	91.3±4.3	479.5± 7.8	128.8±3.0
	10 min	7.39±0.01	33.5±2.4	93.3±3.1	505.0±20.2	121.3±4.5
	45 min	7.36±0.01	33.4±2.0	96.0±3.8	471.0±28.0	117.5±4.8
DN-1417	Control	7.43±0.01	34.8±0.9	101.8±9.1	490.5±11.6	128.9±2.4
	10 min	7.43±0.02	31.0±0.8 ^{a)}	88.3±7.0	481.5±11.6	121.3±1.3 ^{a)}
	45 min	7.45±0.02	27.1±2.3 ^{a)}	103.3±9.7	472.5±21.5	118.3±0.6 ^{a)}
Pentobarbital	Control	7.41±0.01	34.6±1.5	86.0±2.0	497.5± 9.8	124.3±1.5
	10 min	7.34±0.02 ^{b)}	40.3±2.2	88.3±3.5	447.0±28.8	108.3±3.8 ^{b)}
	45 min	7.39±0.01 ^{a)}	33.9±1.2	91.3±1.4	458.5±28.9	112.5±4.3 ^{a)}
Pentobarbital +DN-1417	Control	7.40±0.01	32.9±0.5	88.0±1.5	472.5±12.1	127.8±3.6
	10 min	7.36±0.01 ^{a)}	36.6±2.9	95.7±7.5	415.5±22.8	113.5±7.0
	45 min	7.40±0.01	33.3±2.0	89.7±4.4	426.0±10.7 ^{a)}	111.5±3.9 ^{a)}
Pentobarbital +TRH	Control	7.43±0.02	34.9±1.5	94.3±0.9	478.5±12.1	119.0±0.6
	10 min	7.40±0.01	36.6±1.8	90.5±5.0	388.5±22.1 ^{a)}	112.0±3.4
	45 min	7.43±0.02	33.1±1.7	95.5±4.2	430.5±6.2 ^{a)}	110.0±3.6 ^{a)}
Pentobarbital +ATMB	Control	7.42±0.03	34.7±2.3	94.0±2.0	448.5± 3.8	123.3±1.4
	10 min	7.36±0.01	40.8±2.0	89.0±2.9	402.0±12.2 ^{a)}	107.0±3.9 ^{b)}
	45 min	7.38±0.02	36.7±2.5	97.3±3.5	444.0±13.6	112.5±3.6 ^{a)}
Pentobarbital +ATMB +DN-1417	Control	7.42±0.01	33.9±0.5	92.3±2.2	480.0±17.1	125.0±1.8
	10 min	7.34±0.02 ^{a)}	42.9±3.5 ^{a)}	85.8±4.4	450.0±15.7	106.3±5.9 ^{a)}
	45 min	7.39±0.01	35.2±1.5	88.3±2.8	424.5±20.1	103.0±3.0 ^{c)}
Pentobarbital +PMZ	Control	7.40±0.02	34.8±1.2	94.2±7.1	489.0± 7.1	123.5±1.2
	10 min	7.38±0.01	38.7±1.3	90.6±3.5	377.5±25.1 ^{b)}	110.3±3.9 ^{a)}
	45 min	7.40±0.02	36.2±1.8	94.7±3.7	396.0±26.9 ^{a)}	110.8±3.9 ^{a)}
Pentobarbital +PMZ +DN-1417	Control	7.43±0.01	36.8±0.6	92.5±2.3	483.0±12.6	124.0±5.1
	10 min	7.38±0.02	41.2±0.9 ^{b)}	82.8±0.6 ^{b)}	456.0±17.7	114.3±3.4
	45 min	7.42±0.01	36.9±2.0	89.2±2.9	426.0±16.8 ^{a)}	115.0±2.9

All values are the mean±S.E.M. of 4 rats. Pimozide (PMZ, 1 mg/kg, i.p.) was administered 4 hr before and ATMB (20 µg, i.c.v.) was administered 5 min before the injection of pentobarbital (30 mg/kg, i.v.). Pentobarbital (30 mg/kg, i.v.) and DN-1417 (5 mg/kg, i.v.) or TRH (10 mg/kg, i.v.) were injected 20 min and 5 min before the saline instead of [¹⁴C]DG administration, respectively. Statistically significant level: ^{a)} P<0.05, ^{b)} P<0.01 vs. control.

body, thalamus dorsomedial nucleus, pontine gray matter, sensory-motor cortex and so on. In these structures, non-specific actions also may be involved due to the recovery of the righting reflex by DN-1417 during the 45 min interval after initiating the [¹⁴C]DG infusion.

Kalivas and Horita reported that the most sensitive site in the analeptic action of TRH against pentobarbital-induced sleep was the lateral septum and suggested that TRH modulates neuronal function in the

cholinergic septal-hippocampal pathway via the septum (25, 26). The increase in LCGU by itself and the reversal effect of DN-1417 against pentobarbital in the septal nucleus may support the above speculation.

Miyamoto et al. elucidated the neuro-anatomical substrates mediating the shortening action of TRH or DN-1417 on pentobarbital-induced sleep using a intracerebral microinjection technique in rats (27). The most sensitive sites were the posterior hypothalamic regions including the dorsal

premamillary nucleus, lateral hypothalamic area and posterior nucleus of hypothalamus. Importance of cholinergic mechanisms was suggested in the effects of TRH or DN-1417 in these structures, but not in the septal nucleus. These results coincided with the present findings on LCGU in the hypothalamus and mamillary body.

Pentobarbital reduces the turnover rate of acetylcholine (ACh) in the cerebral cortex (28), hippocampus (29), and hypothalamus (30) due to the inhibition of choline uptake. Narumi et al. showed that TRH or DN-1417 reversed [3 H]-choline uptake reduction induced by pentobarbital and stimulated the conversion of [3 H]-choline to [3 H]-ACh in the slices of rat cerebral cortex, hippocampus, striatum and diencephalon (31). Yarbrough et al. reported the TRH antagonized the reduction in sodium dependent high affinity choline uptake induced by pentobarbital (32). As high affinity choline uptake mechanisms are energy dependent (33, 34), all these results coincide with the present findings that DN-1417 or TRH reverses the LCGU reduction induced by pentobarbital in the hippocampus, caudate-putamen, cerebral cortex and thalamus dorsomedial nucleus via cholinergic mechanisms. On the other hand, dopaminergic mechanisms may not be involved in the effect of DN-1417 or TRH because pimozide did not block the antagonistic effect of DN-1417 against pentobarbital in LCGU. The results are in line with the fact that pimozide did not block the shortening effect of DN-1417 or TRH on pentobarbital-induced sleep (27).

Pentobarbital dramatically reduced blood pressure and heart rate immediately after the injection, but the levels reverted to normal within a few minutes when the LCGU experiment was started. Pentobarbital may reduce glucose transfer into the brain due to these effects on general circulation (35), but the reduction is revised to some extent

by the lumped constant (0.483) which Sokoloff introduced for thiopental on LCGU (15). Thus, these changes in physiological parameters probably do not substantially alter the results of LCGU.

In conclusion, DN-1417 by itself increased the LCGU in the septal nucleus, mamillary body and thalamus dorsomedial nucleus and reversed the reduction of LCGU induced by pentobarbital in the hypothalamus, septal nucleus, hippocampus, mamillary body, thalamus dorsomedial nucleus, and pontine gray matter, probably via a reticulo-thalamo-cortical system. A cholinergic mechanism is responsible for the antagonistic effect of DN-1417 on pentobarbital in LCGU, but a dopaminergic mechanism may not be involved in it.

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