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Effects of R(−)-1-(Benzo[b]thiophen-5-yl)-2-[2-(N,N-diethylamino) ethoxy]ethanol Hydrochloride (T-588), a Novel Cognitive Enhancer, on Noradrenaline Release in Rat Cerebral Cortical Slices

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ABSTRACT. We investigated the effects of R(−)-1-(benzo[b]thiophen-5-yl)-2-[2-(N,N-diethylamino) ethoxy]ethanol hydrochloride (T-588), a novel cognitive enhancer, on noradrenaline (NA) release from rat cerebral cortical slices *in vitro*. Addition of T-588 in an assay mixture stimulated [^{3}H]NA release from prelabeled slices in the presence or absence of extracellular $CaCl_2$, and in the presence of the Ca^{2+}/calmodulin antagonists N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide and trifluoperazine. T-588 stimulated NA release with a time lag of about 1 min, and the high level of release was maintained for at least 10 min, whereas maximal KCl-evoked NA release was observed within 1 min after the addition of KCl, and the effect declined subsequently. The effect of T-588 was reversible (pretreatment with T-588 showed no effect on NA release after two washes by centrifugation). We also compared the effects of T-588 and N-ethylmaleimide (NEM), a sulfhydryl alkylating agent known to stimulate neurotransmitter release in several types of cells. The addition of NEM stimulated NA release irreversibly from the slices in a Ca^{2+}-independent manner, and the effect of NEM, but not that of T-588, was inhibited by the simultaneous addition of dithiothreitol, a sulfhydryl group reducing agent. The addition of T-588, which stimulated NA release by itself, inhibited the NA release by 0.6 mM NEM, although the effect of T-588 was additive in the presence of 0.2 mM NEM. These findings suggest that T-588 stimulates NA release from rat cerebral cortical slices in a Ca^{2+}- and calmodulin-independent manner, possibly via an NEM-sensitive factor(s), although the mechanism of the effects of T-588 seems to be different from that of NEM. BIOCHEM PHARMACOL 53;9:1263–1269, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. T-588; noradrenaline release; cerebral cortical slices; N-ethylmaleimide; Ca^{2+}/calmodulin

T-588§ (Fig. 1) is a novel cognitive enhancer. We previously reported amelioration of learning and memory impairment by T-588 in rats [1–3]. T-588 was found to improved significantly the scopolamine-induced disruption of spatial cognition [1], and to enhance the correct responses in a two-way shuttle box active avoidance task in cerebral-embolized rats produced by injecting microspheres into the internal carotid artery [3]. T-588 also was shown to improve the working memory deficit following cerebral ischemia in rats, as determined using a three-panel runway task apparatus [2]. These effects show that T-588 functions as a cognitive enhancer. We also reported the effects of T-588 on normobaric hypoxia, histotoxic anoxia by KCN, and complete ischemia by decapitation in mice [4]. T-588 significantly enhanced the prolonged survival times in these models, and these effects of T-588 were inhibited by scopolamine, an anticholinergic agent. These findings suggested that T-588 might enhance the cholinergic functions of the central nervous system. However, the effect of T-588 on the gasping duration time following decapitation was not inhibited by scopolamine [4].

Several investigators have reported relationships between catecholamines and hypoxia, anoxia, ischemia, and cognition [5–8]. Moran *et al.* [9] also reported an interaction between central noradrenergic and cholinergic systems in learning and memory processes. Recently, it was suggested that the noradrenergic neurotransmitter system me-

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§ *Abbreviations:* T-588, R(−)-1-(benzo[b]thiophen-5-yl)-2-[2-(N,N-diethylamino)ethoxy]ethanol hydrochloride; NA, noradrenaline; NEM, N-ethylmaleimide; TFP, trifluoperazine; DTT, dithiothreitol; W-7, N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide; and BAPTA-AM, 1,2-bis(2-aminophenoxy)ethane-N,N,N,N-tetraacetic acid.

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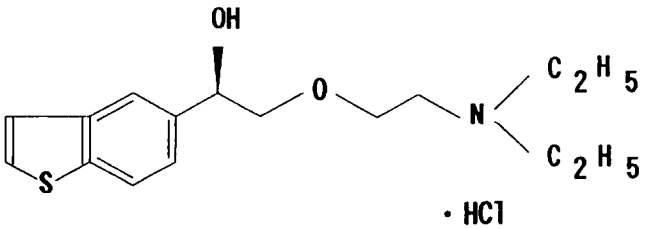

FIG. 1. Chemical structure of T-588.

diates favorable effects on various forms of amnesia [10], and that NA may exert a regulatory influence on the activity of hippocampal cholinergic neurons [11]. Therefore, NA is believed to play an important role in the enhancement of brain functions.

In an *in vivo* microdialysis study, oral administration of 3 or 10 mg/kg T-588 to rats significantly increased acetylcholine and NA overflow in cortical and hippocampal dialysates [12]. Accordingly, it was assumed that T-588 might enhance not only cholinergic function but also other systems, such as the noradrenergic system, by stimulating neurotransmitter release in the brain. In this study, we investigated the effects of T-588 on NA release from rat cerebral cortical slices *in vitro*. We also discuss the possibility of the involvement of an NEM, a sulfhydryl alkylating agent, -sensitive factor(s).

MATERIALS AND METHODS

Materials

T-588, the chemical structure of which is shown in Fig. 1, was synthesized in our laboratory. NEM, TFP, and DTT were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). W-7 hydrochloride was purchased from Research Biochemical Inc. (Natick, MA, U.S.A.) BAPTA-AM and other materials were obtained from the Wako Pure Chemical Corp. (Osaka, Japan). L-[7,8-^{3}H]NA (39 Ci/mmol) was purchased from the Amersham Corp. (Buckinghamshire, U.K.).

Preparation of Rat Cerebral Cortical Slices, Radiolabeling with [^{3}H]NA, and Pretreatment with Agents

Rat cerebral cortical slices were prepared essentially according to the method described by Nomura *et al.* [13]. Male Wistar rats (Japan SLC Inc., Hamamatsu, Japan), weighing 200–250 g, were decapitated. The cerebral cortex of each rat was removed rapidly and was sliced at 400 μm intervals by two passes, following filtration through nylon mesh (300 × 300 μm). The slices were washed twice with modified Tyrode's HEPES buffer (134 mM NaCl, 3 mM KCl, 1 mM Na_2HPO_4, 12 mM $NaHCO_3$, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM glucose, and 20 mM HEPES, pH 7.0) followed by centrifugation at 100 *g* for 15 sec. The Tyrode's buffer was oxygenated by bubbling with O_2 gas before use. The slices were labeled with 50 nM [^{3}H]NA (10 μCi) for 20 min at 37° in Tyrode's HEPES buffer (pH 7.0) containing 10 μM nialamide (monoamine oxidase inhibitor), 20 U/mL of aprotinin (protease inhibitor), and 0.3 mM phenylmethylsulfonyl fluoride (serine protease inhibitor). The pH of the buffer used for the labeling reaction was lowered to avoid degradation of NA. The labeled slices were washed three times with ice-cold Tyrode's HEPES buffer (pH 7.4) in the absence of $CaCl_2$ and $MgCl_2$ by centrifugation at 100 *g* for 15 sec. In some cases, the slices were treated with 5 mL of Tyrode's HEPES buffer (pH 7.4) containing agents such as Ca^{2+}/calmodulin antagonists or NEM after radiolabeling. Subsequently, they were washed three times with the Tyrode's HEPES buffer (pH 7.4) by centrifugation at 100 *g* for 15 sec, and the washed slices were finally resuspended in ice-cold Tyrode's HEPES buffer (pH 7.4) without $CaCl_2$ and $MgCl_2$.

Measurement of [^{3}H]NA Release

Slice suspensions (100–200 μg protein/tube, 200 μL) were incubated for 8 min at 37° in reaction mixture (in total volume, 300 μL) containing 0.2% bovine serum albumin with or without the test compounds. The final composition in the assay mixture was the Tyrode's buffer (pH 7.4) described above. The reaction was terminated by the addition of ice-cold Ca^{2+}- and Mg^{2+}-free modified Tyrode's buffer (500 μL) containing 5 mM EDTA and 5 mM EGTA, followed by centrifugation at 200 *g* for 30 sec. The radioactivity in the supernatant (500 μL) was estimated using a liquid scintillation spectrometer. Total incorporated [^{3}H]NA in the slices was 20,000–30,000 dpm/tube at the end of each experiment. Data are presented as percentages of total incorporated [^{3}H]NA. Protein content was determined using a Bio-Rad assay kit with bovine serum as a standard. Statistical assessment of the data was made by Student's *t*-test. *P* values that were less than 0.05 were considered to be significant.

RESULTS

We investigated the effects of T-588 on [^{3}H]NA release from prelabeled rat cerebral cortical slices. Table 1 shows the effects of T-588 on [^{3}H]NA release from rat cerebral cortical slices in the presence or absence of extracellular $CaCl_2$. T-588 stimulated [^{3}H]NA release in a concentration-dependent manner in both the presence and absence of extracellular $CaCl_2$. The EC_{50} (concentration required to achieve 50% of maximal effect) values in the presence and absence of extracellular $CaCl_2$ were 3.4 ± 0.4 mM (N = 6) and 2.8 ± 0.4 mM (N = 5), respectively, although it was calculated that 10 mM T-588 produced the maximal effect because cell toxicity was induced at more than 10 mM T-588. In contrast, only a limited effect of KCl was observed in the absence of extracellular $CaCl_2$. This procedure measuring [^{3}H]NA release is well established in neuronal tissues [14, 15], and an increase of the tritium is composed predominantly of intact NA, not its metabolites

TABLE 1. Effect of T-588 on [^{3}H]NA release from rat cerebral cortical slices in the presence or absence of extracellular $CaCl_2$

Additions	Concentration (mM)	[^{3}H]NA release (% of total) 1 mM $CaCl_2$	Ca^{2+}-free, 0.2 mM EGTA
None		12.8 ± 0.6	6.1 ± 2.0
T-588	0.3	10.5 ± 1.1	6.6 ± 1.0
	1	15.1 ± 1.4	14.5 ± 1.6*
	3	23.9 ± 1.7*	25.4 ± 2.1†
	10	39.2 ± 3.9†	45.0 ± 4.6†
KCl	20	37.3 ± 1.9†	13.0 ± 3.1

The labeled slices were incubated for 8 min at 37° with T-588 or KCl in the presence or absence of 1 mM $CaCl_2$. EGTA (0.2 mM) was also added to the Ca^{2+}-free buffer. Data are presented as means ± SEM of four to six experiments. Addition of 10 mM T-588 gave the maximal release.

*† Statistical analysis of effects of T-588 were compared with vehicle alone (none) by Student's *t*-test: *$P < 0.05$, and †$P < 0.01$.

[16]. Additionally, there were no differences in T-588-stimulated or KCl-evoked NA release in the presence and absence of the monoamine oxidase inhibitor nialamide in assay mixtures (data not shown). Addition of nialamide (10 μM) also had no effect on the total accumulation of [^{3}H]NA. Therefore, we studied NA release in assay mixtures without the monoamine oxidase inhibitor, except during labeling with [^{3}H]NA.

T-588-stimulated [^{3}H]NA release was not inhibited by various receptor antagonists or inhibitors, i.e. atropine (anticholinergic agent, 10 μM), propranolol (β-blocker, 10 μM) or (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine hydrogen maleate (MK-801; *N*-methyl-D-aspartate receptor antagonist, 10μM) (data not shown). Thus, NA release by T-588 seemed not to involve receptor-mediated cellular depolarization to activate voltage-dependent Ca^{2+} channels resulting in Ca^{2+} influx. Figure 2 shows the effect of T-588 on [^{3}H]NA release from slices pretreated with BAPTA-AM, an intracellular Ca^{2+} chelator. T-588 also stimulated [^{3}H]NA release from BAPTA-AM-pretreated slices in the absence of extracellular $CaCl_2$. These results indicated that alterations in intracellular free Ca^{2+} are not responsible for NA release by T-588. Figure 3 shows the effects of T-588 on [^{3}H]NA release from slices pretreated with W-7 or TFP, Ca^{2+}/calmodulin antagonists, in the absence of extracellular $CaCl_2$. The effect of T-588 was not inhibited by W-7 or TFP pretreatment, nor by pretreatment with both BAPTA-AM (50 μM) and W-7 (50 μM) in the absence of extracellular $CaCl_2$ (data not shown). However, the effect of KCl in the presence of 1 mM $CaCl_2$ was inhibited slightly, but not significantly, by W-7 and was inhibited significantly by TFP. These findings indicate that T-588 may stimulate NA release from rat cerebral cortical slices in an intracellular Ca^{2+} and Ca^{2+}/calmodulin-independent manner.

The labeled slices were incubated with T-588, KCl, or vehicle for the indicated periods, and the effect of T-588 on the time course of [^{3}H]NA release was compared with that evoked by KCl (Fig. 4A). The maximal response to KCl was observed within 1 min after the addition of KCl, and the effect declined subsequently. However, T-588 stimulated [^{3}H]NA release with a time lag of about 1 min, and the high level of release was maintained for at least 10 min (Fig. 4B). In addition, the effect of 20 mM KCl was further enhanced by 3 mM T-588 (data not shown). These findings show that T-588 may stimulate NA release by processes that are different from induction of depolarization such as that produced by KCl.

The effect of T-588 seemed to be reversible, because pretreatment with this agent showed no effect on [^{3}H]NA release after two washes (Fig. 5). This showed that [^{3}H]NA release induced by T-588 was not simply the result of cytotoxicity, and that T-588 is not bound strongly to the affected sites.

Next, we compared the effects of T-588 and the sulfhydryl alkylating agent NEM. Addition of 0.4 mM NEM also stimulated [^{3}H]NA release by about 36% in both the presence (data not shown) and absence of extracellular $CaCl_2$ (Table 2). The effect of NEM was inhibited significantly by the addition of 5 mM DTT, a sulfhydryl group reducing agent. However, there was no effect of DTT on T-588-stimulated [^{3}H]NA release. The stimulatory effect of KCl

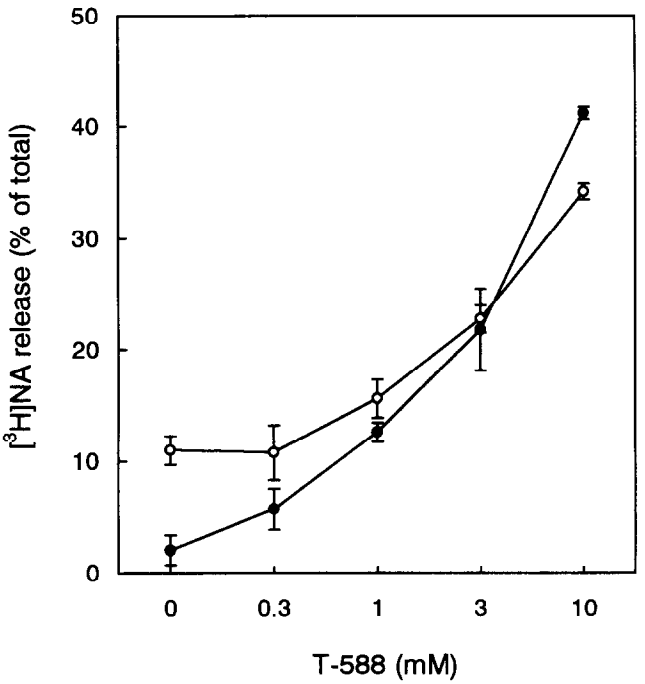

FIG. 2. Effect of T-588 on [^{3}H]NA release from slices pretreated with BAPTA-AM. The labeled slices were incubated for 20 min at 37° with 50 μM BAPTA-AM in Ca^{2+}-free HEPES buffer, and then were washed twice with ice-cold Ca^{2+}-free HEPES buffer. The slices were incubated for 8 min at 37° with the indicated concentrations of T-588 in the presence (○) or absence (●) of extracellular 1 mM $CaCl_2$. Data are means ± SD of two independent experiments performed in triplicate.

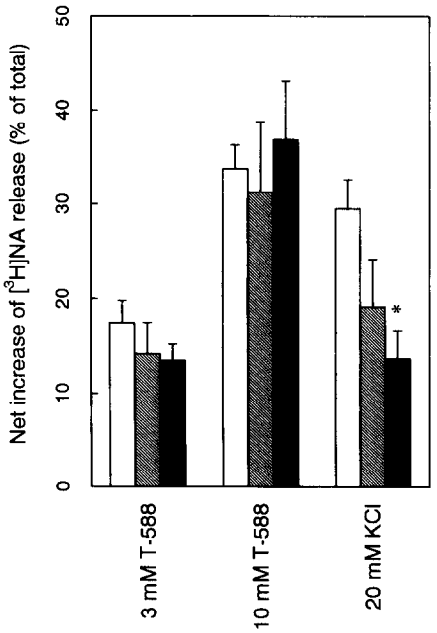

FIG. 3. Effect of treatment with W-7 or TFP on T-588-stimulated [^{3}H]NA release. The labeled slices were incubated with 50 μM W-7 (hatched bars), 50 μM TFP (closed bars) or vehicle (white bars) for 5 min at 37°. W-7 or TFP was added to the suspension again after two washes, and then the slices were incubated for 8 min at 37° for assay of [^{3}H]NA release in the presence of the same concentrations of inhibitors. Data are expressed as net increase percentages of [^{3}H]NA release by the stimulant, and as means ± SEM of three to six independent experiments performed in triplicate. Key: (*) significantly different ($P < 0.05$) from vehicle (none) by Student's *t*-test.

on [^{3}H]NA release in the presence of 1 mM $CaCl_2$ was not modified by DTT. Accordingly, the effect of T-588 does not seem to be via a mechanism similar to that of sulfhydryl selective alkylation agents such as NEM. Table 3 shows the effect of NEM on NA release in the presence of T-588. Addition of 0.3 mM T-588 to the assay mixture, which stimulated [^{3}H]NA release by itself, reduced the effect of 0.6 mM NEM from 55.0 ± 6.4 to 37.5 ± 1.1%. The stimulatory effect of 0.6 mM NEM in the presence of 1 mM T-588 was smaller than that in the absence of 1 mM T-588, although T-588 at this concentration stimulated [^{3}H]NA release 23.6 ± 1.1% by itself. These findings show that T-588 may enhance NA release via an NEM-sensitive factor(s) or protein(s), although the mechanism of NA release evoked by T-588 seems to be different from that induced by NEM.

DISCUSSION

T-588 has been reported to have neuroprotective effects in the central nervous system *in vivo* [1–4]; T-588 significantly improved the working memory deficit following cerebral ischemia and the disruption of spatial cognition induced by scopolamine. On the other hand, 9-amino-1,2,3,4-tetrahydroacridine (tacrine or THA) is an acetylcholinesterase inhibitor that has been reported to be of therapeutic benefit to some patients with clinically diagnosed Alzheimer-type dementia [17]. Many effects of tacrine on learning and memory [18] and cognition [19] have also been reported. In our previous studies, however, tacrine (0.3 to 3 mg/kg, p.o.) was not found to be as effective as T-588 against the ischemia-induced working memory deficit in rats or in improving correct responses in the two-way shuttle box active avoidance task in rats [3]. Additionally, it has been shown that tacrine (5 mg/kg, i.p.) stimulates the release of acetylcholine [20], but not of monoamines [21], in *in vivo* microdialysis studies in rats. These observations have led to the suggestion that T-588 may have other pharmacological properties responsible for the ameliorative ef-

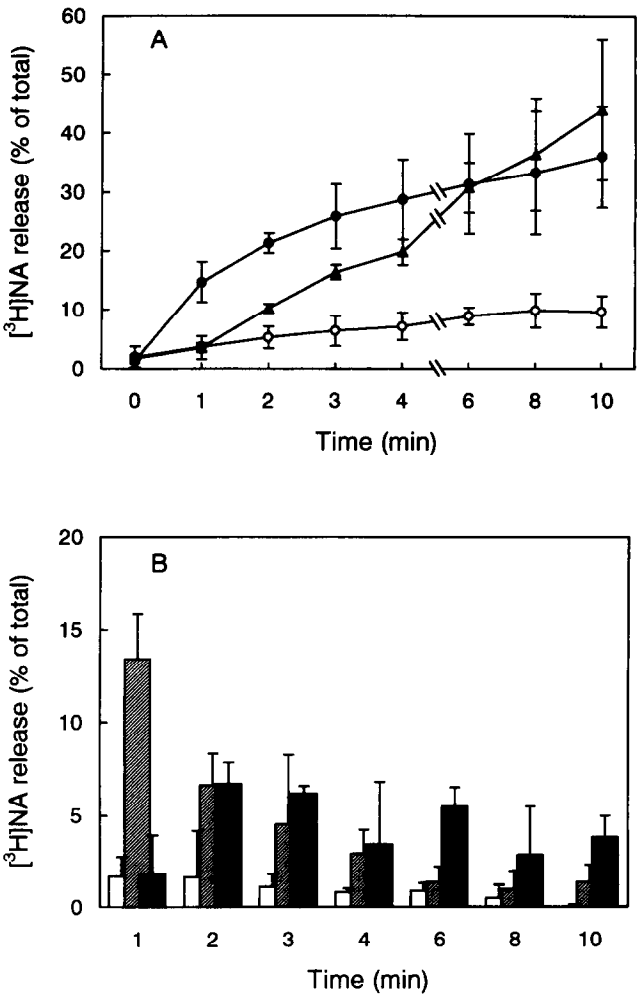

FIG. 4. Comparison of the time courses of T-588-induced and KCl-evoked stimulation of [^{3}H]NA release. In panel A, time courses of spontaneous (○), 20 mM KCl- (●) or 10 mM T-588-(▲) induced stimulation of [^{3}H]NA release for the indicated periods at 37° are shown. Data are means ± SD of two independent experiments performed in duplicate. In panel B, bars (spontaneous, opened bars; 20 mM KCl, hatched bars; 10 mM T-588; closed bars) show the amount of [^{3}H]NA release within each period of 1 min, calculated from panel A. Data are means ± SD of two independent experiments performed in duplicate.

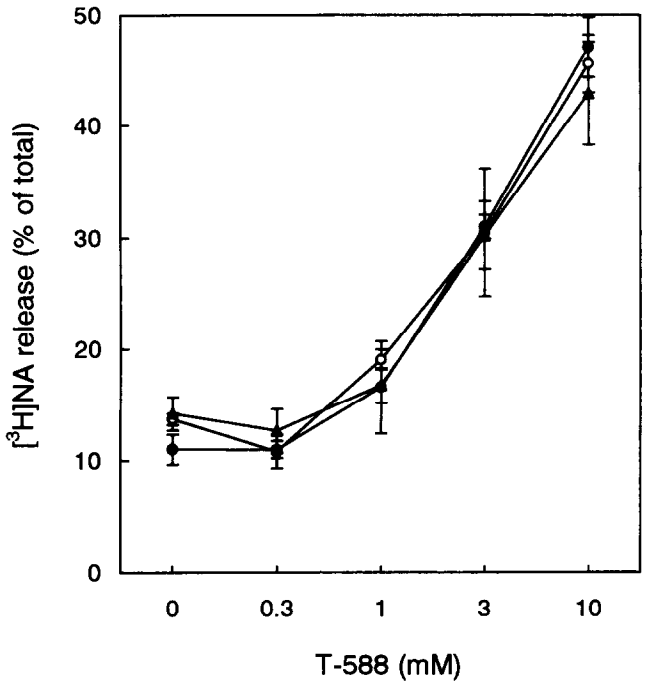

FIG. 5. Effect of T-588 pretreatment on [^{3}H]NA release. The slices were incubated with 1 mM (●) or 3 mM (▲) T-588 or vehicle alone (○) for 10 min at 37°. The washed slices were stimulated with the indicated concentrations of T-588. Data are presented as means ± SD of a typical experiment performed in triplicate, and are representative of two independent experiments.

fects in these models. It has been reported that the adrenoceptors in that area of the cortex were most critical for spatial working memory, and suggested that noradrenergic mechanisms play an important role in the functioning of the frontal cortex [22]. In this study, therefore, we investigated the effects of T-588 on NA release in rat frontal cortical slices *in vitro*.

We previously reported that oral administration of T-588 (3 or 10 mg/kg) to rats significantly increases NA, but not metabolites of NA such as normetanephrine or 3-methoxy-4-hydroxyphenylglycol, overflow from hippocampal dialysates in *in vivo* microdialysis studies [12]. In the present study, addition of T-588 to the assay mixture stimulated [^{3}H]NA release from prelabeled cerebral cortical slices in a concentration-dependent manner (Table 1). Moreover, addition of 3 mM T-588 stimulated [^{3}H]NA release in the presence of 10 μM desipramine, which inhibited NA uptake completely at this concentration (data not shown). In contrast, the stimulatory effect of NA release by tyramine (10 μM), an NA releaser that is known to act on the NA transporter, was inhibited from 37.4 ± 1.6 to 21.4 ± 2.5% ($P < 0.01$, Student's *t*-test, N = 4) by 10 μM desipramine. Therefore, T-588-induced NA release seemed not to involve the transporter of NA. We also confirmed that T-588 increases NA release in both *in vivo* and *in vitro* studies.

The stimulatory effect of T-588 was observed in the absence of extracellular $CaCl_2$ (Table 1) and even in those slices pretreated with BAPTA-AM (Fig. 2). On the other hand, removal of extracellular $CaCl_2$ prior to depolarization resulted in the loss of KCl-induced NA release. It has been reported that Ca^{2+}/calmodulin-dependent protein kinase may play a central role in Ca^{2+}-stimulated catecholamine secretion [23–25]. Gadbut *et al.* [26] reported that angiotensin release evoked by KCl from primary cultures of fetal rat brain is inhibited by W-7. In our experiments, NA release by KCl was inhibited by W-7 or TFP (Fig. 3), although the effect of W-7 was not significant, as described previously [27]. Addition of W-5, which has many nonspecific effects without inhibition of calmodulin, did not inhibit the effect of KCl (data not shown). These findings suggest that the effect of KCl-evoked depolarization may be partially modulated in a Ca^{2+}/calmodulin-dependent manner. However, T-588 enhanced NA release from slices pretreated with W-7 or TFP in the absence of extracellular $CaCl_2$ (Fig. 3). Accordingly, these results suggest that T-588 enhanced the NA release independently of extracellular or intracellular Ca^{2+}, and in a Ca^{2+}/calmodulin-independent manner.

NA release evoked by KCl (20 mM) showed the maximal response within 1 min after stimulation of depolarization as described previously [28, 29]. However, T-588 stimulated NA release with a time lag of about 1 min, and T-588 gradually enhanced NA release and maintained the high level for at least 10 min (Fig. 4). This result suggested that T-588 may interact with an as yet undetermined factor(s) or protein(s), although the effect of T-588 was reversible. The effect of T-588 did not seem to be derived from cell toxicity or an increase in permeability of cell membranes, because no stimulatory effect was observed in slices that were preincubated with T-588 and then washed by centrifugation (Fig. 5).

TABLE 2. Effect of DTT on T-588- or NEM-induced [^{3}H]NA releases

Additions	Concentration (mM)	[^{3}H]NA release (% of total) None	[^{3}H]NA release (% of total) 5 mM DTT
Experiment I (Ca^{2+}-free)			
None		4.8 ± 1.5	6.4 ± 0.4
T-588	3	22.9 ± 2.5	24.6 ± 1.8
	10	35.5 ± 1.1	41.7 ± 2.2
NEM	0.2	19.3 ± 1.8	11.7 ± 2.0*
	0.4	36.4 ± 2.8	10.8 ± 2.9†
Experimental II (1 mM $CaCl_2$)			
None		13.7 ± 0.7	15.4 ± 1.3
KCl	20	35.3 ± 0.6	34.4 ± 1.5

The labeled slices were incubated for 10 min at 37° with or without 5 mM DTT. The washed slices were incubated for 8 min at 37° with the indicated agents in the absence (Experiment I) or presence (Experimental II) of 1 mM CaC_2. Five millimolar DTT or vehicle was also added to the assay mixture. Data are presented as means ± SEM of three independent experiments.

*† Significantly different from vehicle alone (without DTT) by Student's *t*-test: *$P < 0.05$, and †$P < 0.01$.

TABLE 3. Effect of NEM on [^{3}H]NA release under coexistence with T-588

Additions	Concentration (mM)	[^{3}H]NA release (% of total) None	0.3 mM T-588	1 mM T-588
None		6.2 ± 1.8	13.3 ± 3.0	23.6 ± 1.1
NEM	0.2	19.8 ± 1.8	24.7 ± 1.7	29.6 ± 1.7
	0.6	55.0 ± 6.4	37.5 ± 1.1	42.4 ± 1.0

The labeled slices were incubated for 8 min at 37° with the indicated concentrations of NEM in the absence of extracellular $CaCl_2$. The assay mixture was further supplemented with 0.3 or 1 mM T-588 or vehicle. Data are presented as means ± SD of a typical experiment performed in triplicate, and are representative of three independent experiments.

Next, we compared the effects of T-588 and NEM. There have been several reports on the effects of NEM on catecholamine secretion or machinery of release of other neurotransmitters in several cell types [30, 31]. Addition of NEM to the assay mixture stimulated [^{3}H]NA release from rat cerebral cortical slices in a concentration-dependent manner (Table 2). The stimulatory effect of NEM was observed in the absence of extracellular $CaCl_2$. In the presence of 0.3 mM T-588, which stimulated NA release by itself, the effect of 0.6 mM NEM was decreased (Table 3). In the presence of 1 mM T-588, the effect of NEM was small, although the addition of 1 mM T-588 stimulated NA release to about 24%. Additionally, 0.6 mM NEM by itself increased NA release by about 49%. However, the increase of NA release by NEM was only about 24 and 19% in the presence of 0.3 and 1 mM T-588, respectively. Therefore, the effect of 0.6 mM NEM was inhibited in the presence of T-588. In addition, in slices pretreated with 0.2 or 0.4 mM NEM, T-588 (0.3 to 3 mM) had no effect (data not shown). These findings suggest that T-588 may stimulate NA release via an NEM-sensitive factor(s) or protein(s). We found that the effect of NEM was inhibited by the simultaneous addition of DTT (Table 2), but the effect of T-588 was not. Similar inhibitory actions of DTT on the effects of NEM have been reported previously [32, 33]. The effect of NEM was irreversible, but that of T-588 seemed to be reversible. Accordingly, T-588 may enhance NA release through a process other than alkylation of sulfhydryl groups.

In conclusion, we found that T-588 enhanced NA release by a mechanism distinct from that involving internal calcium levels, and might be mediated via an NEM-sensitive factor(s) or protein(s). However, in the present study using cerebral cortical slices, the EC_{50} value of T-588 was much higher than that previously reported *in vivo* [12]. Further consideration is needed on this point. We hope that T-588 will become useful as a novel cognitive enhancer in the therapy of demential disorders through stimulation of neurotransmitter release in the brain.

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