Pharmacology Biochemistry and Behavior, Vol. 42, pp. 693-698, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Effects of Tizanidine on Morphine Physical Dependence: Attenuation and Intensification

H. KOYUNCUOĞLU,[1] F. ARICIOĞLU, Y. ÜRESIN, Y. DİZDAR AND Y. ESİN

Istanbul Medical Faculty, Department of Pharmacology and Clinical Pharmacology, 34390 Çapa, Istanbul, Turkey

Received 17 September 1991

KOYUNCUOĞLU, H., F. ARICIOĞLU, Y. ÜRESIN, Y. DİZDAR AND Y. ESİN. *Effects of tizanidine on morphine physical dependence: Attenuation and intensification.* PHARMACOL BIOCHEM BEHAV 42(4) 693-698, 1992. – It has previously been shown that subchronic and acute administration of L-asparaginase and glutaminase inhibitors D-Aspartic acid (D-ASP) and prolyl-leucyl-glycinamide (PLG) intensifies and attenuates morphine (M) physical dependence, respectively, by the inhibition of ASP and glutamic acid (GLU) production, and subsequently their normal releases. Tizanidine (TIZ) has long been known to be an α_2-adrenoceptor agonist and inhibitor of ASP and GLU release. Therefore, in this study TIZ has been administered subchronically during the development of M physical dependence to rats in which M-containing pellets had been implanted or acutely 30 min before naloxone (NL)-induced abstinence syndrome. The subchronic administration of TIZ intensified NL-precipitated abstinence syndrome whereas its acute administration attenuated it, as did D-ASP and PLG. On the other hand, TIZ added into the medium prevented the in vitro M-dependent-made guinea pig ileum from contracting following NL application. Furthermore, TIZ stopped the already started contraction by NL of the M-dependent ileum, which completely relaxed later. These effects of TIZ on M-dependent ileum were antagonized by the α_2-adrenoceptor antagonist yohimbine. The intensification by subchronic TIZ administration of abstinence syndrome was attributed to the lesser release of ASP and GLU, which resulted in the larger blockade of M of ASPergic/GLUergic receptors due to the lesser release of their endogenous agonist ASP and GLU and consequently the higher upregulation of the receptors. The attenuation by acute TIZ administration of NL-precipitated abstinence syndrome was explained with lesser release of ASP and GLU and concomitantly the lesser stimulation of the receptors. The results of the in vitro experiments were considered supporting evidence for the fact that α_2-adrenoceptor agonistic effect of TIZ causes the inhibition of ASP and GLU release and for this action TIZ has already been combined with dextromethorphan in the treatment of heroin addicts.

Tizanidine Yohimbine Intensification of morphine dependence Attenuation of morphine abstinence syndrome Inhibition of aspartate/glutamate release α_2-Adrenoceptor

THE hypothesis (25,29,30) in relation to the probable mechanisms underlying the physical dependence upon and tolerance to opiates implies a) the inhibition by opiates of L-asparaginase and glutaminase, the enzymes producing excitatory amino acid (EAA) neurotransmitters aspartate (ASP) and glutamate (GLU) in the nerve ends of their systems from asparagine and glutamine (6,44); b) the blockade by opiates of the ASPergic/GLUergic receptors and the upregulation and probable supersensitivity of these receptors due to the blockade of the receptors (26); c) the centrally and peripherally prompt adaptation of the organism to the lesser production of the neurotransmitters ASP and GLU and the blockade, upregulation, and supersensitivity of the ASPergic/GLUergic receptors. In case of withdrawal from opiates, the normalization of the production of ASP and GLU and their release will occur and these EAA's, to a greater extent, stimulate ASPergic/GLUergic system receptors that are not under the blocking effect of opiate anymore. The stimulation of the ASPergic/GLUergic receptors results in the release of some other neurotransmitters and hormones, such as acetylcholine (ACh), adrenaline, noradrenaline, dopamine, corticotropin, gonadotropins, growth hormone, etc., each of which has long been considered a responsible candidate for the abstinence syndrome. Consistent with the hypothesis, it has already been shown that the administration of the inhibitors of the enzymes such as D-ASP (27,32) or prolyl-leucyl-glycinamide (PLG) (27) prior to naloxone (NL) administration attenuated the intensity of the precipitated abstinence syndrome in rats due to the inhibition of ASP and GLU production (28). It has also been shown that the administration of D-ASP or PLG during the development of morphine (M) physical dependence intensified NL-induced abstinence syndrome due to less production of ASP and GLU (28). Again in agreement with the hypothesis, when NMDA, one of the subtypes of the ASPergic/GLUergic

[1] To whom requests for reprints should be addressed.

receptors, was blocked by its noncompetitive blockers ketamine or dextromethorphan, the NL-elicited abstinence syndrome was markedly inhibited or completely suppressed (29). On the basis of these experimental results, dextromethorphan has successfully been used in the treatment of heroin addicts for more than 3 years (25,30,46).

The existence of a direct relationship between α_2-adrenoceptors in rat CNS and chronic M administration has long been known (18). The α_2-adrenoceptor agonist clonidine has been used effectively to reduce withdrawal symptoms (13,56,59). In addition, clonidine has been reported to possess an antinociceptive action (36) that is reversed by yohimbine, an α_2-adrenoceptor antagonist (58), and to significantly reduce the force of the contracture induced by NL in the isolated guinea pig ileum taken from M-dependent guinea pig (4,8). The latter method and its modified form, which implies the use, following exposure to opioids, of isolated guinea pig ileum taken from naive animals, have been considered useful as a model to test acute effects of opioids in vitro (9,24,41,49) and investigate some characteristics of opioid-induced physical dependence and tolerance in vitro (17,33,34,40,50,53,57). Because the opioid receptors of the myenteric plexus show characteristics similar to those in the CNS and the neuronal elements of the myenteric plexus closely resemble those of the CNS anatomically and neurochemically (9,11,16,19,24), when the guinea pig ileum or longitudinal muscle myenteric plexus preparation is stimulated electrically, ACh is released as it occurs in the CNS (41,42). ACh is also released in the NL-induced abrupt contracture of opioid-dependent isolated guinea pig ileum (15,51). In accordance with the information given above, M reduces the output of ACh and in association with this inhibits the contracture by electrical stimulation of isolated guinea pig ileum (9,41). But, there is no evidence to indicate that M acts on the cholinergic terminals. Furthermore, methionine-enkephalin also inhibits electrically induced contraction of the longitudinal muscle (21) and sensitizes the isolated guinea pig ileum to NL (34). On the other hand, it is known that adrenaline, isoprenaline, and dopamine suppress the electrically induced contracture in isolated guinea pig ileum (17), most probably via a decrease in ACh output in the preparation. Moreover, it has recently been reported that ASPergic/GLUergic receptors and especially their subtype NMDA receptors exist in the myenteric plexus of the guinea pig (35,37,52). In the absence of Mg^{+2}, L-GLU, L-ASP, or D-GLU produce a rapid contraction of the isolated guinea pig ileum (52). The contraction has been found to be antagonized by the competitive NMDA antagonists (D-2-amino-5-phosphovalerate (APV) and 3-(±-2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), as well as by the noncompetitive antagonists gamma-D-glutamylglycine (GDGG), glutamic acid diethyl ester (GDEE), and the phencyclidine-like drugs etoxadrol, dextromethorphan, and 5-methyl-10, 11-dihydroxy-5H-dibenzo (*a, b*) cyclohepten-5, 10-imine (MK-801) showing that the stimulation of NMDA receptors induces the contraction. In addition, the GLU-induced contraction is blocked by tetrodotoxin and hyoscine, indicating that the stimulation of NMDA receptors induces contractions of the longitudinal muscle via the ACh release from nerve endings in the myenteric plexus (52). This is consistent with the other experimental findings in relation to the ACh release by the ASPergic/GLUergic receptors (3,5,31,43,48,54). Moreover, the NMDA stimulation-dependent release of ACh has been reported to be antagonized by opioids and noncompetitive NMDA receptor antagonists (5,54).

DS 103-282, 5-chloro-4-(2-imidazolin-2-yl-amino)-2, 1,3-benzothiazole hydrochloride [tizanidine (TIZ)], whose chemical structure is similar to that of clonidine, has been reported to have antinociceptive activity as well as selective α_2-adrenoceptor agonistic and centrally acting muscle relaxant effects (10,22,38,39,47,55). The inhibition of synthesis of noradrenaline, dopamine, and 5-hydroxytryptamine, the manipulation of dopaminergic and serotonergic systems with neurotoxins, and the depletion by reserpine of monoamines have been found ineffective in modifying the action of TIZ (39). Instead, the blockers of α-adrenoceptors can significantly decrease the antinociceptive action of TIZ. The decreasing effect of yohimbine, a selective blocker of the α_2-adrenoceptors, is markedly stronger than nonselective ones (39) and the selective binding of [^{3}H]yohimbine to α_2-adrenoceptors has been inhibited by TIZ and clonidine (55). In addition to these, TIZ has been reported to inhibit spontaneous movements of in situ guinea pig ileum and rat stomach and intestinal transit in mice and induce mydriasis in mice. All these actions might be due to the activation of α_2-adrenoceptors but not to atropine-like action (55). However, the actions of TIZ, which have been given above, may be related to its stimulating effects of presynaptically located α-$_2$-adrenoceptors in the central ASPergic/GLUergic systems that inhibit the release of EAA neurotransmitters ASP and GLU subsequently (12) because the activation of NMDA receptors causes the release of ACh, as mentioned above (3,5,31,43,48,52,54), as well as the release of some other neurotransmitters and hormones.

As a result of the experimental findings given so far, it is possible to say that TIZ can be used to provide more support for the above-mentioned probable mechanisms underlying the development of physical dependence upon and tolerance to opioids (25,26,29,30,46), the inhibitory effect of TIZ on the release of ASP and GLU within their systems, and the reason for the contracture due to the release of ACh after NL in the in vitro preparation of guinea pig ileum made opiate dependent by incubating in opiate containing Tyrode solution as described before (17,33,34,40,50,53,57). Therefore, TIZ has been administered to rats in which M-containing pellets were implanted during the development of physical dependence or just before NL-precipitated abstinence syndrome to see the probable intensifying effect of TIZ administered during the development and the probable attenuating effect of TIZ given just before abstinence syndrome. The different administration schedule of TIZ in these experiments was suggested by the uses of D-ASP and PLG, which, instead of inhibiting the release of ASP and GLU, inhibit the production of ASP and GLU, which intensify or attenuate the severity of physical dependence when given during the development or before NL-induced abstinence syndrome, respectively (28). In addition, the guinea pig ileum segments that were made M dependent by preincubation with M-containing Tyrode solution had been treated with TIZ before NL addition into the medium to see whether TIZ could prevent the NL-induced contraction of the M-dependent isolated guinea pig ileum. If TIZ could prevent the NL-induced contraction, it would mean that an abstinence syndrome sign derived from the antagonism by NL of the M-induced NMDA receptor blockade can be abolished by the stimulation of α_2-adrenoceptors located presynaptically at the ASPergic/GLUergic system. To show that the prevention by TIZ of the contraction is due to α_2-adrenoceptor stimulation, which decreases the release of ASP and/or GLU, the selective α_2-adrenoceptor blocker yohimbine also has been added into the medium before the TIZ application in the separate experiments.

METHOD

Experiments on Rats to Investigate the Probable Intensifying Action of Subchronic TIZ Administration During Development of M Physical Dependence and the Probable Attenuating Action of Single-Dose TIZ Just Before NL-induced Abstinence Syndrome

Rats were divided into three groups. The second group [chronic tizanidine group (CTG)] was injected with 0.2 mg/kg TIZ IP. The other two groups received the same volume of physiological saline IP. One hour later, two pellets containing 75 mg base M (total 150 mg) were SC implanted in the back of all rats under light ether anesthesia (60). Seven hours after pellet implantation, CTG was IP given again 0.2 mg/kg TIZ whereas the other two groups IP received physiological saline. On the second and third day of implantation, the dose of TIZ augmented to 0.3 mg/kg and was given twice a day with an interval of 8 h. On the morning of the fourth day, rats belonging to the third group [acute tizanidine group (ATG)] were IP injected with 0.3 mg/kg TIZ; the first (control) and CTG groups were IP given physiological saline instead. Thirty minutes after IP administration of TIZ or physiological saline, all rats were injected with 2 mg/kg NL IP. Immediately after NL administration, rats were placed in a metal cage (base area 20 × 22 cm, height 20 cm) and strictly observed. The number of jumps, wet-dog shakes, and defecations were counted for 15 min. Diarrhea and ptosis were rated 1, 2, or 3, whereas teeth-chattering was rated 1–10 in accordance with their intensity. In addition, on the basis of Himmelsbach's Degree Method (20), which characterizes the abstinence syndrome into four grades to reflect the clinical severity and the correlations among the occurrence, onset and fading of each abstinence syndrome signs according to the M content of the implanted pellet(s), the exposure of animals to different M content pellet(s), and the amount of NL administered to precipitate abstinence syndrome (7), each sign was rated as follows. Every jump, wet-dog shake, maximum degree of teeth-chattering, diarrhea, defecation, the ptosis were separately scored 8, 4, 5, 10, 1, and 3, respectively. To give overall information of the six signs for each group, the total score of each group was shown as a total evaluation of the abstinence syndrome intensity. The precipitated abstinence syndrome was induced only once in each rat. All results were first analyzed by one-way analysis of variance (ANOVA); subsequently, the statistical evaluation was carried out by Student's *t*-test. $p < 0.05$ was considered statistically significant.

Experiments To See the Effects of TIZ on the Contractions by NL Application of the M-Dependent Isolated Guinea Pig Ileum

The terminal portion of the ileum was obtained from male inbred guinea pigs weighing 300–400 g, fasting for 24 h, and killed by cervical dislocation. After the intestine was nicely and thoroughly washed in Tyrode solution ($NaCl_2$ 8.0 g, KCl 0.2 g, $CaCl_2$ 0.2 g, $NaHCO_3$ 1.0 g, NaH_2PO_3 0.05 g, glucose 2.0 g, choline chloride 0.929 mM) by flushing Tyrode solution through the lumen, it was cut into segments of 5 cm. Then, the segments were placed in Tyrode solution containing 1 μM M and kept at 4°C for 4 h (34,40). Then, the segments that had been made M dependent (34,40) were fixed at a resting tension of 1 g and kept for 1 h prior to any drug application. During the equilibration of 1 h and also throughout the experiments, the tissue was washed out with 1 μM M-containing Tyrode solution warmed at 37°C and continuously bubbled with 95% O_2 and 5% CO_2. At the end of the equilibration period, NL, TIZ, and yohimbine were applied in the solutions of 0.1 ml provided their concentrations of each drug should be 1, 1, and 10 μM, respectively. The intervals of multiple drug applications were 10 min. Each segment was tested only once and the contractions were recorded by means of a Nihon-Kohden SB-1T Force-Displacement Transducer, Nihon-Kohden RM-150 Polygraph (Tokyo, Japan), Acer 1120 SX Personal Computer, PC LAB PCL 718 A-D Converter (Taiwan, Taiwan), Labtech Acquire Data Acquisition Programme, and Lotus Data Analysis Programme. Each category that represents a different combination of drugs was repeated at least five times and every time the results of each category were in the same direction.

Materials

Male Wistar inbred rats (weighing 140–180 g) and male inbred guinea pigs (weighing 300–400 g) kept in a room 22–23°C on a 12 L : 12 D cycle and fed with a standard regimen ad lib were used. TIZ was a gift from Turkish Sandoz (Istanbul, Turkey). NL and M were purchased from Sigma Chemical Co. (St. Louis, MO) and Verenigde Pharmaceutische Fabrieken B. V. (Holland), respectively.

RESULTS

The mean values (±SE) and their statistical evaluations of the results obtained from the experiments performed on rats treated with TIZ during the development of M physical dependence or just before NL injection are shown in Table 1. The mean values of jumping, wet-dog shake, teeth-chattering, defecation, and ptosis were found significantly lower in ATG than those in controls, whereas the values of these signs were significantly higher in CTG than those in both controls and ATG.

In Fig. 1, all individual graphs, which were differentiated from each other by being shown with A, B, C, D, and E, represent the other four ones similar to each other. The NL-evoked contraction of the isolated guinea pig ileum that was previously incubated with M and made M dependent can be seen in Fig. 1A. When 1 μM TIZ concentration is provided in the medium 10 min before NL application, contraction does not occur (Fig. 1B). If the quantity of TIZ for providing 1 μM concentration in the medium is added on the contracting M-dependent isolated guinea pig ileum following NL application, the already started contraction stops and a complete relaxation appears (Fig. 1C). The addition of yohimbine 10 min before TIZ allows NL application to elicit contraction of the M-dependent isolated guinea pig ileum despite the presence of TIZ in the medium (Fig. 1D). The NL-evoked contraction of the M-dependent isolated guinea pig ileum in the presence of previously added yohimbine in the medium is not affected by the addition of TIZ (Fig. 1E).

DISCUSSION

It has long been accepted that a statistically significant decrease in the "dominant" abstinence syndrome signs – flying, jumping, teeth-chattering, defecation, and ptosis – and a significant increase in "recessive" sign – wet-dog shake – indicate a less severe M physical dependence development. Instead, the significant increase in the dominant abstinence syndrome

TABLE 1

MEAN VALUE (±SE) OF THE ABSTINENCE SYNDROME SIGNS AND THEIR STATISTICAL EVALUATIONS

Signs	Groups			ANOVA
	Control (13)	Acute Tizanidine (13)	Chronic Tizanidine (14)	
Jumping	8.15 ± 0.43	4.68 ± 0.30*	19.64 ± 0.84*†	$F = 197.635, p < 0.05$
Wet-dog shake	2.38 ± 0.27	0.39 ± 0.21*	1.64 ± 0.23*†	$F = 20.379, p < 0.05$
Teeth-chattering	4.69 ± 0.17	3.23 ± 0.27*	8.21 ± 0.44*†	$F = 72.487, p < 0.05$
Diarrhea	0.54 ± 0.22	0.31 ± 0.18	0.50 ± 0.20	$F = 0.428, p < 0.05$
Defecation	5.69 ± 0.26	4.85 ± 0.19*	6.86 ± 0.28*†	$F = 18.442, p < 0.05$
Ptosis	1.54 ± 0.15	0.84 ± 0.26*	2.36 ± 0.13*†	$F = 17.338, p < 0.05$
Total evaluation of signs	114.07	66.98	223.61	

The figures in parentheses indicate the number of rats in each group.
*Significance referring to control values.
†Significance between acute tizanidine (ATG) and chronic tizanidine (CTG) groups.

signs and significant decrease in the recessive sign reflect a more intense development of M physical dependence (4).

The results presented in Table 1 clearly show that the administration of TIZ 30 min before NL injection attenuates the intensity of the NL-precipitated abstinence syndrome. This attentuation can normally be attributed to the inhibition of ASP and GLU release due to the stimulation by TIZ of α_2-adrenoceptors probably located at the presynaptic part of the ASPergic/GLUergic system. The lesser release of ASP and GLU leads to the lesser stimulation of upregulated and supersensitive ASPergic/GLUergic receptors and subsequently to the lesser release of other neurotransmitters and hormones that are responsible for the manifestation of abstinence syndrome. When ASP- and GLU-producing enzymes are inhibited by D-ASP or PLG before NL-induced abstinence syndrome, the production and release of ASP and GLU are also reduced. As a result, ASPergic/GLUergic receptors are also less stimulated (28). When rats in which M-containing pellets had been implanted received TIZ (CTG) during the development of M physical dependence, the inhibition of ASP and GLU release occurred. If the hypothesis implying that opiates block ASPergic/GLUergic receptors works, the lesser release of ASP and GLU due to the effect of TIZ can create a much more favorable condition for opiate than controls in compet-

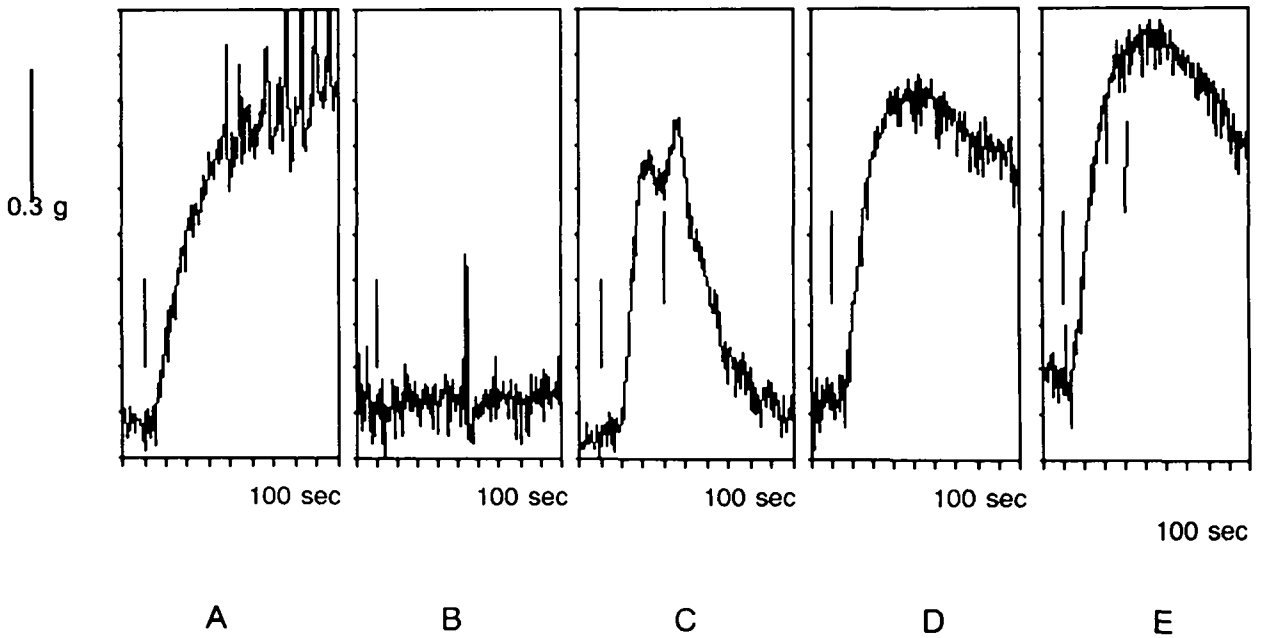

FIG. 1. Naloxone (NL), tizanidine (TIZ), and yohimbine concentrations were 1, 1, and 10 μM, respectively. (A) Contraction by NL of the guinea pig ileum segment made morphine (M) dependent after incubation in M-containing Tyrode solution. (B) Antagonism by TIZ added into the medium 10 min prior to NL application of the NL-induced contraction in the M-dependent guinea pig ileum segment. (C) Abolition by TIZ of the NL-induced contraction. (D) Failure of TIZ in antagonizing the NL-induced contraction of the M-dependent guinea pig ileum pretreated with yohimbine before TIZ addition. (E) Failure of TIZ in antagonizing the NL-induced contraction of the M-dependent guinea pig ileum pretreated with yohimbine before NL-induced contraction.

ing for the blockade of ASPergic/GLUergic receptors against their endogenous agonists ASP and GLU. This, without a doubt, leads to a much larger blockade by opiate of the receptors and consequently to a much larger compensatory upregulation and supersensitivity of the receptors. The same consideration may be valid in the case of chronic administration of D-ASP or PLG, both of which decrease the production of ASP and GLU (28). The antinociceptive action of TIZ may also be explained on the basis of this decreased stimulation of ASPergic/GLUergic receptors. Since the stimulation of the receptors increases the transmission and perception of pain, and the blockade by competitive NMDA receptor antagonists of the receptors increases analgesic threshold (1,2,23,45), it is possible that the determination of pain perception underlies the balance or better competition between NMDA receptor agonists and antagonists. The antinociceptive activity of D-ASP, which decreases ASP and GLU production (H. Koyuncuðglu, unpublished observation), and competitive NMDA receptor antagonist CGS 19755 (14) may be considered supporting evidence. As seen in Fig. 1A, NL addition into the M-containing Tyrode solution, where the segment of guinea pig ileum previously incubated in M-containing Tyrode solution at 4°C for 4 h to make it M dependent (33,34,40,50,57) had been mounted, evoked a prompt contraction of the ileum. This contraction was due to the release of ACh (15,41,42,51) because the inhibitory effect of M on the release of ACh (9,41) was abolished by NL. Since NMDA receptors exist in the myenteric plexus (35,37,52), and L-ASP and L-GLU cause the contraction of isolated guinea pig ileum, which is antagonized by anticholinergic drugs and NMDA receptor antagonists (52), and the stimulation of ASPergic/GLUergic receptors induces ACh release (3,5,31,43,48,54), the inhibition by opioids (15,21,34) of the isolated guinea pig ileum contractions would be associated with the blockade by opioids of NMDA receptors as previously assumed (25,29,30). Having reached this point, the explanation of the TIZ-induced prevention (Fig. 1B) of the NL-elicited M-dependent guinea pig ileum contraction underlies the inhibitory effect of TIZ on the release of ASP and GLU (12) via the stimulation of α_2-adrenoceptors because the stimulation of NMDA receptors causes ACh release (3,5,31,43,48,52,54). When TIZ was added into the M-dependent guinea pig ileum contracted by NL application, the contraction was completely antagonized (Fig. 1C). The relaxant effect of TIZ on the contracting ileum clearly shows that continuous ACh release promptly stops because TIZ starts to stimulate α_2-adrenoceptors at the ASPergic/GLUergic systems without any competition. The addition of TIZ into the M-dependent guinea pig ileum whose α_2-adrenoceptors were previously blocked by the selective α_2-adrenoceptor blocker yohimbine (39) before (Fig. 1D) or after (Fig. 1E) NL application cannot prevent contraction of the guinea pig ileum. These latter experiments also show that 10 μM concentration of yohimbine easily prevents α_2-adrenoceptors from being stimulated by 1 μM concentration of TIZ. Since almost all the adrenergic system agonists, such as adrenaline, isoprenaline, dopamine, etc., stimulate α_2-adrenoceptors to different extents, the well-known action of the adrenergic system in relation to the inhibition of some gastrointestinal functions is probably attributed to the inhibition of ACh release due to the stimulation by the adrenergic neurotransmitters of α_2-adrenoceptors (17), as does TIZ.

Finally, it should be added that TIZ has begun to be used together with dextromethorphan, chlorpromazine, and anticholinergic and analgesic drugs (25,30) in the treatment of heroin addicts and it has been observed that TIZ is very helpful in shortening and diminishing the intensity of the abstinence syndrome signs (H. Koyuncuðglu, unpublished observations).

REFERENCES

1. Aanonsen, L. M.; Lei, S.; Wilcox, G. L. Excitatory amino acid receptors and nociceptive neurotransmission in rat spinal cord. Pain 41:309-321; 1990.
2. Aanonsen, L. M.; Wilcox, G. L. Nociceptive action of excitatory amino acids in the mouse: Effects of spinally administered opioids, phencyclidine and sigma agonists. J. Pharmacol. Exp. Ther. 243:9-19; 1987.
3. Alberch, J.; Sanchez-Arroyos, R.; Solsona, C.; Marsal, J. Amino acid regulation of acetylcholine release in the neurostriatum. Neurosci. Lett. 26 (suppl.):s151; 1986.
4. Alfaro, M. J.; Colado, M. I.; Lopez, F.; Martin, M. I. Effect of clonidine, nimodipine and ditiazem on the in vitro opioid withdrawal response in the guinea pig ileum. Br. J. Pharmacol. 101: 958-960; 1990.
5. Arenas, E.; Alberch, J.; Sanchez-Arroyos, R.; Marsal, J. Effect of opioids on acetylcholine release evoked by K+ or glutamic acid from rat neostriatal slices. Brain Res. 523:51-56; 1990.
6. Bielarczyk, H.; Lysiak, W.; Szutowicz, A. Synthesis of glutamate and aspartate in rat brain synaptosomes. Acta Biochim. Pol. 33: 239-251; 1986.
7. Blaesig, J.; Herz, A.; Reinhold, K.; Zielgaensberger, S. Development of physical dependence on morphine in respect to time and dosage and quantification of the precipitated withdrawal syndrome in rats. Psychopharmacologia 33:19-38; 1973.
8. Collier, H. O. J.; Cuthbert, N. J.; Francis, D. L. Clonidine dependence in guinea pig isolated ileum. Br. J. Pharmacol. 73:443-453; 1981.
9. Cox, B. M.; Weinstock, M. The effect of analgesic drugs on the release of acetylcholine from electrically stimulated guinea pig ileum. Br. J. Pharmacol. Chemother. 27:81-92; 1966.
10. Coward, D. M.; Davies, J.; Herrling, P.; Ruedeberg, C. Pharmacological properties of tizanidine (DS 103-282). In: Conrad, B.; Benecke, R.; Bauer, H. J., eds. Die klinische wertung der spastisitaet. Stuttgart: Schattaer Press; 1984:61-69.
11. Creese, I.; Snyder, S. H. Receptor binding and pharmacological activity of opiates in the guinea pig intestine. J. Pharmacol. Exp. Ther. 194:205-219; 1975.
12. Davies, J.; Johnston, S. E.; Lovering, R. Inhibition by DS 103-282 of D-[^{3}H] aspartate release from spinal cord slices. Br. J. Pharmacol. 78(proc. suppl.):2P; 1983.
13. Fielding, S.; Wilker, J.; Hynes, M.; Szewczak, M.; Novick, W. J., Jr.; Lal, H. A. Comparison of clonidine with morphine for antinociceptive and anti-withdrawal actions. J. Pharmacol. Exp. Ther. 207:899-905; 1978.
14. France, C. P.; Winger, G. D.; Woods, J. H. Analgesic, anesthetic, and respiratory effects of the competitive *N*-methyl-D-aspartate (NMDA) antagonist CGS 19755 in rhesus monkeys. Brain Res. 526:355-358; 1990.
15. Frederickson, R. C. A.; Hewes, C. R.; Aiken, J. W. Correlation between the in vivo and in vitro expression of opiate withdrawal precipitated by naloxone: Their antagonism by 1-(-)-Δ-tetrahydrocannabinol. J. Pharmacol. Exp. Ther. 199:375-384; 1986.
16. Gershon, M. D.; Bursztajn, S. Properties of the enteric nervous system: Limitation of access of intravascular macromolecules to the myenteric plexus and muscularis externa. J. Comp. Neurol. 180:467-488; 1978.
17. Goldstein, A.; Schultz, R. Morphine-tolerant longitudinal muscle strip from guinea pig ileum. Br. J. Pharmacol. 48:655-666; 1973.
18. Hamburg, M.; Tallman, J. F. Chronic morphine administration

increases the apparent number of alpha-2 adrenergic receptors in rat brain. Nature 291:493; 1981.
19. Harris, L. S.; Dewey, W. L.; Howes, J. F.; Kennedy, J. S.; Pars, H. Narcotic antagonist analgesics: Interactions with cholinergic system. J. Pharmacol. Exp. Ther. 169:17-22; 1969.
20. Himmelsbach, C. K. Addiction liability of codeine. JAMA 103: 1420-1423; 1934.
21. Hughes, J.; Smith, T. W.; Kosterlitz, H. W.; Fothergill, L. A.; Morgan, B. A.; Morris, H. R. Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature 258:577-579; 1975.
22. Kameyama, T.; Nabeshima, T.; Sugimoto, A.; Matsuno, K.; Yamada, S. Antinociceptive action of tizanidine in mice and rats. Naunyn-Schmied. Arch. Pharmacol. 330:93-96; 1985.
23. Klepstad, P.; Maurset, A.; Moberg, E. R.; Oye, I. Evidence of a role for NMDA receptors in pain perception. Eur. J. Pharmacol. 187:513-518; 1990.
24. Kosterlitz, H. W.; Waterfield, A. A. In vitro models in the study of structure-activity relationships of narcotic analgesics. Annu. Rev. Pharmacol. 15:29-47; 1975.
25. Koyuncuğlu, H. The treatment with dextromethorphan of heroin addicts. In: Loimer, N.; Schmid, R.; Springer, A., eds. Drug addiction and AIDS. Vienna: Springer Verlag; 1991:320-329.
26. Koyuncuğlu, H.; Ariciğlu, F. Previous chronic blockade of NMDA receptors intensifies morphine-dependence in rats. Pharmacol. Biochem. Behav. 39:575-579; 1991.
27. Koyuncuğlu, H.; Berkman, K.; Matthaei, H. Effects of morphine, D-aspartic acid, D-phenylalanine, D-leucine and PLG on L-asparaginase activity in rats. Med. Bull. Istanbul 17:1-6; 1984.
28. Koyuncuğlu, H.; Güngör, M.; Săgduyu, H.; Ariciğlu, F. Intensification and attenuation of morphine dependence by D-aspartic acid and PLG. Pharmacol. Biochem. Behav. 35:47-50; 1990.
29. Koyuncuğlu, H.; Güngör, M.; Săgduyu, H.; Ariciğlu, F. Suppression by ketamine and dextromethorphan of precipitated abstinence syndrome in rats. Pharmacol. Biochem. Behav. 35: 829-832; 1990.
30. Koyuncuğlu, H.; Saydam, B. The treatment of heroin addicts with dextromethorphan: A double-blind comparison of dextromethorphan with chlorpromazine. Int. J. Clin. Pharmacol. Ther. Toxicol. 28:147-152; 1990.
31. Lehmann, J.; Scatton, B. Characterization of the excitatory amino acid receptor-mediated release of [^{3}H]-acetylcholine from rat striatal slices. Brain Res. 252:77-89; 1982.
32. Lerman, M. I.; Verevkina, I. V. The inhibition of asparaginase from the blood serum of guinea pigs. Biokhimiya 27:526-531; 1962.
33. Lujan, M.; Rodriguez, R. Further characterization of opiate physical dependence in the isolated guinea pig ileum. Pharmacologist 20:268; 1978.
34. Lujan, M.; Rodriguez, R. Pharmacological characterization of opiate dependence in the isolated ileum of the guinea pig. Br. J. Pharmacol. 73:859-866; 1981.
35. Luzzi, S.; Ziletti, L.; Franchi-Michelli, A. M.; Moroni, F. Agonist, antagonist and modulators of excitatory amino acid receptors in the guinea pig myenteric plexus. Br. J. Pharmacol. 95: 1271-1277; 1988.
36. Milne, B.; Cervenco, F. W.; Jhamandas, K.; Sutak, M. Intrathecal clonidine: Analgesia and effect on opiate withdrawal in the rat. Anesthesiology 62:34-38; 1985.
37. Moroni, F.; Luzzi, S.; Franchi-Micheli, A. M.; Ziletti, L. The presence of *N*-methyl-D-aspartate-type receptors for glutamic acid in the guinea pig myenteric plexus. Neurosci. Lett. 68:57-62; 1986.
38. Nabeshima, T.; Matsuno, K.; Kameyama, T. Involvement of spinal and supraspinal structures in tizanidine-induced antinociceptive action. Neurosci. Lett. 68:1-4; 1986.
39. Nabeshima, T.; Matsuno, K.; Sugimoto, A.; Kameyama, T. Antinociceptive activity induced by tizanidine and alpha-2 adrenoreceptors. Neuropharmacology 26:1453-1455; 1987.
40. Opmeer, F. A.; van Ree, J. M. Quantification of the in vitro induced tolerance to morphine of the isolated guinea pig ileum. Neuropharmacology 17:887-890; 1978.
41. Paton, W. D. M. The action of morphine and related substances on contraction and on acetlycholine output of coaxially stimulated guinea pig ileum. Br. J. Pharmacol. Chemother. 11:119-127; 1957.
42. Paton, W. D. M.; Zar, M. A. The origin of acetylcholine released from guinea pig intestine and longitudinal muscle strips. J. Physiol. (Lond.) 194:13-33; 1968.
43. Ranson, R. W.; Deschenes, N. L. Glycine modulation of NMDA-evoked release of [^{3}H]-acetylcholine and [^{3}H]-dopamine from rat striatal slices. Neurosci. Lett. 96:323-327; 1989.
44. Reubi, J. C.; Toggenburger, G.; Cuenod, M. Asparagine as precursor for transmitter aspartate in corticostriatal fibers. N. Neurochem. 34:1015-1017; 1980.
45. Rusin, K. I.; Randic, M. Modulation of NMDA-induced currents by μ-opiate receptor agonist DAGO in acutely isolated rat spinal dorsal horn neurons. Neurosci. Lett. 124:208-212; 1991.
46. Saydam, M. B.; Koyuncuğlu, H. Dextromethorphan-treatment of heroin addicts: A double-blind clinical trial. The Abstract Book of VIIIth World Congress of Psychiatry, Athens, Greece, October 12-19, 1989, abstr. 19.
47. Sayers, A. C.; Burki, H. R.; Eichenberger, E. The pharmacology of 5-chloro-4-(2-imidazolin-2-yl-amino)2,1,3-benzothiadiazole (DS 103-282), a novel myotonolytic agent. Drug. Res. 30:793-803; 1980.
48. Scatton, B.; Lehmann, J. *N*-Methyl-D-aspartate-type receptors mediate striatal [^{3}H]-acetylcholine release evoked by excitatory amino acids. Nature (Lond.) 297:422-424; 1982.
49. Schultz, R.; Herz, A. The guinea pig ileum as an in vitro model to analyse dependence liability of narcotic drugs. In: Kosterlitz, H. W., ed. Opiates and endogenous opioid peptides. Amsterdam: Elsevier/North-Holland Biomedical Press; 1976:319-326.
50. Schultz, R.; Herz, A. Dependence liability of enkephaline in the myenteric plexus of the guinea pig. Eur. J. Pharmacol. 39:429-432; 1976.
51. Schultz, R.; Seidl, E.; Herz, A. Opioid dependence in the guinea pig myenteric plexus is controlled by non-tolerant and tolerant opioid receptors. Eur. J. Pharmacol. 110:335-341; 1985.
52. Shannon, H. E.; Sawyer, B. D. Glutamate receptors of the *N*-methyl-D-aspartate subtype in the myenteric plexus of guinea pig ileum. J. Pharmacol. Exp. Ther. 251:518-523; 1989.
53. Shoham, S.; Weinstock, M. The role of supersensitivity of acetylcholine in the production of tolerance to morphine in the stimulated guinea pig ileum. Br. J. Pharmacol. 52:597-603; 1974.
54. Snell, L. D.; Johnson, K. M. Antagonism of NMDA-induced transmitter release in the rat striatum by phencyclidine-like drugs and its relationship to turning behavior. J. Pharmacol. Exp. Ther. 235:50-57; 1985.
55. Takayanagi, I.; Konno, F.; Kusunoki, M. Some pharmacological effects of tizanidine on smooth muscle organs and alpha-2 adrenoreceptor. Gen. Pharmacol. 16:501-503; 1985.
56. Taylor, J. R.; Elsworth, J. D.; Garcia, E. J.; Grant, S. J.; Roth, R. H.; Redmond, D. E. Clonidine infusions into the locus coeruleus attenuate behavioral and neurochemical changes associated with naloxone-precipitated withdrawal. Psychopharmacology (Berl.) 96:121-134; 1988.
57. Villareal, J. E.; Martinez, J. N.; Castro, A. Validation of a new procedure to study narcotic dependence in the isolated guinea pig ileum. In: Report of the 39th Meeting of the Committee on the Problems of Drug Dependence. Washington, DC: National Academy of Science; 1977:305-344.
58. Wang, Y. C.; Chain-Fa, S.; Mao-Tsun, L. The site and mode of analgesic actions exerted by clonidine in monkeys. Exp. Neurol. 90:479-485; 1985.
59. Washton, A. M.; Resnick, R. B. Clonidine for opiate detoxification: Outpatient clinical trial. Am. J. Psychiatry 137:1121-1122; 1980.
60. Way, E. L.; Loh, H. H.; Shen, F. Simultaneous quantitative assessment of morphine tolerance and physical dependence. J. Pharmacol. Exp. Ther. 167:1-8; 1969.