POTENTIATION OF CARDIOVASCULAR EFFECTS OF CATECHOLAMINES BY A DIPHENYLALKANOLAMINE

Hisakuni HASHIMOTO* and Yutaka KASUYA

Department of Toxicology and Pharmacology, Research Institute for Chemical Hazards
Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo, Japan

Accepted May 15, 1974

Abstract The mechanisms of the CA-potentiating action of DMA, 1-phenyl-1-(2, 5-dimethoxyphenyl)-3-piperidinobutanol, were investigated in cats. DMA specifically prolonged the duration of the cardiovascular responses to adrenaline and isoproterenol. The responses to noradrenaline were only slightly potentiated. While DMA inhibited the disappearance of intravenously injected adrenaline and isoproterenol from blood plasma, the disappearance of noradrenaline was only slightly inhibited. It is suggested that the inhibitory effect of DMA on the disappearance of adrenaline contributed to the potentiating effect of DMA on the pressor response to adrenaline. The increase of the plasma level of $^3$H-CAs by DMA was not accompanied by any reduction in the total amount of their metabolites. In rabbits, the synergistic effect was observed on the delayed disappearance of plasma $^3$H-adrenaline among DMA, pyrogallol and tranylcypromine. It is concluded that the DMA-sensitive inactivation process of circulating catecholamines is different from the O-methylation or the monoamine oxidation of catecholamines and has a higher affinity for adrenaline and isoproterenol than for noradrenaline.

It is well known that many drugs inhibit the enzymatic inactivation or the tissue uptake of catecholamines (CAs). Cocaine inhibits the neuronal uptake of CAs (1-4). Pyrogallol and tropolones inhibit the O-methylation of CAs (5, 6). It is generally accepted that inhibitions of the inactivations of CAs by these drugs contribute to the potentiation of the pharmacological effects of CAs (7-9).

In a previous paper it was reported that 1-phenyl-(2, 5-dimethoxyphenyl)-3-piperidinobutanol (Dimethoxyasparninol, DMA) potentiated the effects of adrenaline in whole animals (10). DMA prolonged the duration of the pressor response to adrenaline, and this effect of DMA was further enhanced by the pretreatment of animals with a combination of pyrogallol and pheniprazine.

The present investigation was undertaken to study the effect of DMA on the inactivations of CAs and clarify the relationship between the effect and the CA-potentiating capacity.

MATERIALS AND METHODS

Recording of blood pressure, heart rate, respiration and the contraction of the nictitating membrane

Cats of both sexes, weighing 1.8 to 3.4 kg, were anesthetized with pentobarbital (40

*Present Address; Research Institute for Chemobiodynamics, Chiba University, Narashino, Chiba, Japan
mg/kg, i.p.), and cervically vagotomized. The blood pressure was recorded from the femoral artery using a pressure-displacement transducer (Nihon Kohden MP-4T). Heart rate and respiration were recorded with a pulse rate tachometer (Nihon Kohden RT-2) and a pressure-displacement transducer, respectively. The contractions of the nictitating membranes were recorded isometrically with a strain-gauze transducer (Nihon Kohden RP-3). The resting tension on the muscle was 2 g.

The following drugs were used; l-adrenaline • HC1 (0.5-4 µg/kg), l-noradrenaline • HC1 (1-3 µg/kg), dl-isoproterenol • HC1 (0.5-1 µg/kg), DMA • HC1 (4 mg/kg), cocaine • HC1 (4 mg/kg). All drugs were administered intravenously.

Measurement of disappearance of l-adrenaline and l-noradrenaline from blood plasma

Cats were anesthetized with pentobarbital. Since the plasma levels of CAs after i.v. injections varied over a wide range depending on the individual, the experiment was designed as follows: CA was injected twice into each animal at an interval of about three hours. DMA and other drugs were administered at a given time before the 2nd injection of the CA. The plasma levels of the CA after the 2nd injection were expressed in terms of % of the level at one min after the first injection, and the influence of various drugs on the CA levels after the 2nd injection were compared.

Blood samples (3 to 6 ml) were rapidly withdrawn from the femoral artery immediately before and 1 to 3 min after each injection of a CA. DMA • HC1, its analogue YS-3623 (10), or cocaine • HC1, at a dosage of 4 mg/kg, was administered through the femoral vein 20 min before the 2nd injection of a CA. In the control group, one ml of a physiological saline was injected instead of a drug.

CAs were determined according to the method of Vendslau (11). Correction was made for recovery (80%) of CAs.

CAs used were l-adrenaline • HC1 (15 µg/kg) and l-noradrenaline • HC1 (25 µg/kg).

Determination of dl-3H-adrenaline, dl-3H-isoproterenol and their metabolites in blood plasma

Cats of both sexes, or male rabbits weighing 2.0-2.5 kg, were anesthetized with pentobarbital and blood samples were collected 2.5 min after the i.v. injection of 3H-CA. Four mg/kg of DMA • HC1 or the same dose of cocaine • HC1 was administered i.v. 20 min before the 3H-CA injection. Pyrogallol, 50 mg/kg, was administered i.v. 5 min before the injection of 3H-CA. Tranylcypromine, 10 mg/kg, was given s.c. about 20 hours before the CA injection. 3H-CAs used were dl-7-3H-adrenaline • HC1 (10 µg/kg) (dl-7-3H-adrenaline • HC1 with specific activity 10.1 Ci/mM which was obtained from the New England Nuclear Corp. Boston, Mass., U.S.A. was diluted to 1 µC/µg with non-radioactive l-adrenaline • HC1 and dl-7-3H-isoproterenol bitartrate (2 µg/kg) (dl-7-3H-isoproterenol bitartrate with specific activity 0.027 mC/µg which was obtained from the New England Nuclear Corp. was diluted to 2 µC/µg with non-labelled dl-isoproterenol bitartrate).

The extraction and separation of 3H-CA and its metabolites was carried out as follows. The plasma samples were deproteinized by the addition of 1/3 volume of 1.2 N perchloric acid. In the experiment with 3H-isoproterenol, 1 µg of adrenaline was added.
as a carrier to each of the plasma samples. An aliquot of the acid extracts was used to estimate the total $^3$H activity. $^3$H catechols in other aliquots of the acid extracts were adsorbed to 0.6 g of alumina as described by Anton and Sayre (12). The $^3$H-CA was eluted with 12 ml of 0.05 N perchloric acid. Total amount of metabolites were calculated by subtracting the $^3$H-CA in acid eluate of alumina from the total $^3$H. $^3$H-metanephrine or $^3$H-O-methylisoproterenol in the effluent of alumina was determined with Dowex 50 column as described by Kopin et al. (13). Deaminated catechols present in the acid eluate from alumina were determined by washing an aliquot of the acid eluate with ethyl acetate and measuring the radioactivity extracted in the latter (13). This radioactivity was negligible in all samples so treated. The recovery of $^3$H-CA was 85%. Loss of $^3$H-CA occurred mainly during the washing of alumina, and the contamination of $^3$H-CA was negligible in the fractions of metabolites.

In order to determine the radioactivity, 0.3 to 1.5 ml of each fraction was dissolved with 18 ml of p-dioxane phosphor consisting of 10% naphthalene, 0.7% PPO and 0.35% dimethyl POPOP, and counted with a liquid scintillation spectrometer (Packard Tri Carb 3203). Counting efficiency was determined by the channel ratio method (14).

RESULTS

1. Influence of DMA and cocaine on the cardiovascular responses and responses of the nictitating membranes to CAs

Fig. 1 shows the influence of DMA on the responses to adrenaline. The duration of the pressor response to adrenaline was markedly prolonged by DMA. The duration of the chronotropic response was also prolonged and the contraction of the nictitating membrane was markedly enhanced. Fig. 2 shows the influence of DMA on the responses

Fig. 1. Cardiovascular responses to 1-adrenaline (4 µg/kg) before (left tracings) and 20 min after DMA (4 mg/kg) (right tracings) in the cat. Resp.: respiration. B.P.: blood pressure. H.R.: heart rate. Nict. M.: contractions of nictitating membranes.
Fig. 2. Cardiovascular responses to 1-noradrenaline (1 μg/k g) before (left tracings) and 20 min after DMA (4 mg/kg) (right tracings) in the cat.

Fig. 3. Cardiovascular responses to dl-isoproterenol (1 μg/kg) before (left tracings) and 30 min after DMA (4 mg/kg) (right tracings) in the cat.

Fig. 4. Influence of DMA and cocaine on the pressor or depressor responses to CAs in the cat. Each value is the mean of six experiments, and horizontal bars indicate the standard errors of means. DMA : DMA, 4 mg/kg. Cocaine : cocaine, 4 mg/kg. Nor. : noradrenaline, 1 μg/kg. Adr. : adrenaline, 4 μg/kg. Iso. : dl-isoproterenol, 1 μg/kg. Abscissae : the responses after DMA or cocaine, expressed in terms of % of the response before the drugs. The magnitude of the pressor or depressor response was calculated as follows : Response = (maximum height of the response) x (duration of the response), where the duration of the response is the time interval from the initiation of the response and the recovery to one-half of the maximum height of the response.
to noradrenaline. The pressor response was slightly potentiated, and the chronotropic response was inhibited. The depressor response to isoproterenol was slightly inhibited (Fig. 3). However, the duration of the chronotropic response to isoproterenol was markedly prolonged. Cocaine markedly potentiated the responses to noradrenaline. The responses to adrenaline were also potentiated by cocaine, but to a lesser extent than those to noradrenaline, while those to isoproterenol were not potentiated (Fig. 4).

2. Influence of DMA and cocaine on the disappearance of circulating l-adrenaline and l-noradrenaline

Fig. 5 shows the influence of DMA and cocaine on the disappearance of adrenaline. The plasma level of adrenaline at one min after the first injection varied from 61 m\(\mu\)g/ml to 147 m\(\mu\)g/ml. The pre-injection level of plasma CAs was negligible. In the control group (saline-treated group), the plasma levels at 1 to 3 min after the 2nd injection were higher than those levels after the first injection. In the DMA-treated animals, plasma

![Graph showing the influence of DMA and cocaine on the disappearance of adrenaline.](image-url)
levels at various times after the adrenaline injection were higher than the corresponding controls. In DMA-treated animals, the plasma level of adrenaline one min after the 2nd injection was about 2.5 times that of the corresponding control. After 2 min it was about 6 times as much and nearly 10 times as much 3 min after the injection. Thus, DMA evidently delayed the rate of the disappearance of adrenaline. A similar delay of the disappearance was observed in cocaine-treated animals. However, the inhibition of the disappearance by cocaine was to a lesser extent than by DMA. Fig. 6 shows the influence of DMA and cocaine on the disappearance of noradrenaline from plasma. The plasma level of noradrenaline one min after the first injection varied between 36 μg/ml and 103 μg/ml. Cocaine markedly inhibited the disappearance of noradrenaline. Inhibition of the disappearance of noradrenaline by DMA was rather slight. Cocaine thus inhibited the disappearance of noradrenaline more so than it did that of adrenaline, and DMA inhibited the disappearance of adrenaline more markedly than that of noradrenaline.

Fig. 5 shows the influence of a DMA analogue (YS-3623) on the disappearance of adrenaline. In a previous paper (10), it was shown that YS-3623 did not potentiate the pressor response to adrenaline. YS-3623 slightly inhibited the disappearance of adrenaline. Here a causal relationship
between the potentiation of the pressor response and the inhibition of the disappearance is suggested.

3. Influence on the plasma levels of dl-3H-adrenaline and its metabolites

As is shown in Fig. 8, pretreatment with DMA elevated the 3H-adrenaline fraction. In other words, DMA inhibited the disappearance of 3H-adrenaline from blood plasma. All other fractions were also slightly increased by DMA.

The elevation of the plasma level of dl-3H-adrenaline by DMA was much smaller than that of non-radioactive l-adrenaline shown in Fig. 5. For this reason, the effects of DMA on the plasma level of 3H-adrenaline were compared with the effect of cocaine. Administration of cocaine caused no significant increase in the plasma level of 3H-adrenaline, thus, the effect of DMA was greater than that of cocaine, this result being the same as that with non-radioactive adrenaline. The difference in degree of the elevation of the plasma level by DMA may be due to the difference in the stereoisomer of adrenaline used, i.e.; dl-type of 3H-adrenaline and l-type of non-radioactive adrenaline.

![Table 1](image)

**Table 1**. Influence of cocaine, DMA and a DMA analogue on the pressor effect and disappearance from blood plasma of CAs. Adr.; adrenaline. Nor; noradrenaline. + + ; marked potentiation. + ; potentiation. (−) ; slight potentiation. − − ; marked inhibition. − ; inhibition.

![Chart 1](image)

**Fig. 7.** Influence of cocaine, DMA and a DMA analogue on the pressor effect and disappearance from blood plasma of CAs. Adr.; adrenaline. Nor; noradrenaline. + + ; marked potentiation. + ; potentiation. (−) ; slight potentiation. − − ; marked inhibition. − ; inhibition.

**Fig. 8.** Influence of DMA on the plasma levels of dl-3H-adrenaline and its metabolites in the cats. DMA, 4 mg/kg, was administered 20 min before the injection of dl-3H-adrenaline (10 μg/kg). Blood samples were withdrawn 2.5 min after the injection of dl-3H-adrenaline. N = number of animals used. The values are the means ± S.E.M. (horizontal lines). Significance of difference to the saline-treated group; *P<0.05, **P<0.01.
4. Influence of DMA on the levels of $^{3}$H-isoproterenol and its metabolites in blood plasma

DMA markedly elevated the $^{3}$H-isoproterenol in plasma (Fig. 9). $^{3}$H-isoproterenol fraction was increased. $^{3}$H-O-methylisoproterenol fraction and total metabolites fraction were also increased by DMA. Therefore, total $^{3}$H activity in plasma was increased.

5. Influence of DMA on the plasma levels of $^{3}$H-adrenaline and its metabolites in the enzyme inhibitors-pretreated rabbits

A previous paper showed a synergistic effect between DMA, pyrogallol and pheniprazine on the pressor response to adrenaline in rabbits (10). The present experiment was...
designed to determine whether or not there was a synergistic effect between DMA, pyrogallol and tranylcypromine on the elevation of the plasma level of ³H-adrenaline.

Results are shown in Fig. 10. In rabbits, DMA elevated the plasma level of ³H-adrenaline. This elevation was enhanced by a combination of pyrogallol and tranylcypromine. The total metabolites and total ³H activity in plasma were also increased by DMA whether or not the animals were treated with the enzyme inhibitors. The enzyme inhibitors slightly reduced the total metabolites and also slightly increased the ³H-adrenaline level. In comparison with DMA treatment alone, the treatment with a combination of the enzyme inhibitors and DMA increased the ³H-adrenaline level and slightly reduced the total metabolites fraction.

DISCUSSION

Dimethoxyasparminol (DMA) in doses far less than the toxic level potentiated the actions of adrenaline in cats as previously reported (10). In the present investigations it was shown that DMA specifically prolonged the duration of the cardiovascular responses to adrenaline and isoproterenol (Figs. 1, 3 and 4), and that DMA markedly inhibited the disappearance of injected adrenaline and isoproterenol from blood plasma, whereas it inhibited the disappearance of noradrenaline only slightly (Figs. 5, 6 and 9). In contrast, cocaine showed remarkable inhibition of the disappearance of noradrenaline but a weak inhibition in the case of adrenaline (Figs. 5 and 6). These facts suggest that the DMA-sensitive process of the disappearance of CAs is different from the cocaine-sensitive one. As summarized in Fig. 7, a stronger inhibitor of the disappearance potentiated the response, to a much greater extent than did a less potent inhibitor. This suggests that the inhibition of the disappearance of injected CAs contributes to the potentiation of the responses to the CAs by these drugs.

It is possible that DMA inhibits the metabolism of injected CAs, resulting in the elevation of the plasma level of these CAs. The elevation of the plasma level of ³H-adrenaline by DMA, however, was not due to the inhibition of the O-methylation of ³H-adrenaline, as the level of ³H-metanephrine was not reduced by DMA (Fig. 8). In addition, this increase of the level of ³H-adrenaline by DMA was also observed in the presence of pyrogallol (Fig. 10). The plasma level of ³H-isoproterenol was increased without any reduction of the O-methyl metabolites (Fig. 9), also suggesting that the elevation of the level of ³H-isoproterenol was not due to the inhibition of the O-methylation. Since Herting (15) reported that monoamine oxidation does not contribute to the metabolism of isoproterenol, it is unlikely that the elevation of the level of ³H-isoproterenol by DMA is due to the inhibition of the monoamine oxidation.

DMA, also in rabbits, elevated the plasma level of ³H-adrenaline. The elevation of the level of ³H-adrenaline was intensified by the simultaneous administration of the enzyme inhibitors (Fig. 10). This synergistic effect between DMA and the enzyme inhibitors can account for the synergistic effect among DMA, pyrogallol and pheniprazine on the pressor response to adrenaline (10). The treatment with pyrogallol and tranylcypromine, un-
expectedly, did not cause any significant changes in the levels of $^3$H-adrenaline or its metabolites. Axelrod and Tomchic (16) reported that catechol-O-methyltransferase activity in the rabbit liver was lower than that in the rat or the cat liver. Thus it appears that in rabbits, the conjugations in the liver as well as O-methylation play a significant role in the inactivation of circulating adrenaline. It is also possible that increase of the plasma level of $^3$H-adrenaline by the enzyme inhibitors will be compensated by the uptake process, because the inactivation of $^3$H-adrenaline by the enzyme may not be so much as that by the uptake process. In comparison with the treatment with DMA alone, the treatment with a combination of DMA and the enzyme inhibitors increased the level of $^3$H-adrenaline and slightly decreased the level of $^3$H-metabolites, and increase of the total radioactivity was slight. Therefore, it is reasonable to postulate that the decrease in the level of $^3$H-metabolites contributes to the increase in the level of $^3$H-adrenaline in the latter treatment. In other words, inhibition of enzymatic degradation by the enzyme inhibitors contributed to the synergistic effect. In rabbits as well as in cats, the increase of the plasma level of $^3$H-CA by DMA did not accompany any reduction in the level of $^3$H-metabolites, suggesting that the increase of plasma $^3$H-CA level is not due to the inhibition of enzymatic inactivation of $^3$H-CA such as O-methylation, monoamine oxidation, or the conjugations.

The important pathways for the inactivation of CAs besides enzymatic degradations and conjugations are binding and uptake in various tissues (17, 18). According to Iversen (3), CAs are taken up by tissues through two mechanisms, i.e., uptake1 and uptake2, the former is cocaine-sensitive and the latter is cocaine-insensitive. Noradrenaline has a higher affinity for uptake1, whereas isoproterenol and adrenaline have higher affinities for uptake. Hertting (15) showed that the fate of $^3$H-isoproterenol administered intravenously was very different from that of $^3$H-noradrenaline. It seems that DMA-sensitive inactivation process of CAs is a process which has a higher affinity for adrenaline and isoproterenol than for noradrenaline. The effects of DMA on the tissue binding of CAs in various organs are being examined.

REFERENCES
1) Whitby, L.G., Hertting, G., and Axelrod, J.: Nature 187, 604 (1960)
2) Hertting, G., Axelrod, J., and Whitby, L.G.: J. Pharmacol. exp. Ther. 134, 146 (1961)
3) Iversen, L.L.: Br. J. Pharmacol. Chemother. 25, 18 (1965)
4) Iversen, L.L.: Br. J. Pharmacol. Chemother. 37, 627 (1969)
5) Axelrod, J., and Laroch, J.M.: Science 130, 800 (1959)
6) Axelrod, J., Albers, W., and Clement, C.C.: J. Neurochem. 5, 68 (1959)
7) Trendelenburg, U.: J. Pharmacol. exp. Ther. 125, 55 (1959)
8) Hardmann, J.G., and Mayer, S.E.: J. Pharmacol. exp. Ther. 148, 29 (1965)
9) Kasuya, Y., and Goto, K.: Europ. J. Pharmacol. 4, 355 (1965)
10) Kasuya, Y., and Watanabe, M.: Arzneim. Forsch. 15, 1279 (1965)
11) Vendslau, A.: Acta. physiol scand. 49, Suppl. 173 (1960)
12) Anton, A.H., and Sayre, E.D.: J. Pharmacol. exp. Ther. 138, 360 (1962)
13) Kopin, I.J., Axelrod, J., and Gordon, E.: J. Biol. Chem. 236, 2109 (1961)
14) Bailie, L.A.: Interc. J. aplli. Radiat. Isotopes 8, 1 (1960)
15) Hertting, G.: Biochem. Pharmacol. 13, 1119 (1964)
16) Axelrod, J., and Tomchic, R.: J. Biol. Chem. 233, 702 (1958)
17) Iversen, L.L.: Br. J. Pharmacol. Chemother. 41, 571 (1971)
18) Von Euler, U.S.: Catecholamines, Edited by Blaschko, H. and Muscholl, E., p. 186
Springer-Verlag, Berlin, (1972)