PHARMACOLOGIC ANALYSIS OF NICOTINE AND DIMETHYL-PHENYLPIPERAZINIUM ON PACEMAKER ACTIVITY OF THE SA NODE IN THE DOG

Shigetoshi CHIBA, Kiyoshi TAMURA, Katsumi KUBOTA and Koroku HASHIMOTO
Department of Pharmacology, School of Medicine, Tohoku University, Sendai, Japan

Received for publication April 6, 1972

It is well known that nicotine and 1,1-dimethyl-4-phenylpiperazinium (DMPP) have biphasic chronotropic responses both by stimulating the intracardiac parasympathetic ganglia and by liberating catecholamines locally (1-4). In 1967, Nadeau and James reported effects of nicotine on heart rate using a direct perfusion technique of the SA node in dogs (5). They showed that the negative chronotropic response to nicotine was abolished by treatment with atropine and that of the positive by either propranolol or hexamethonium. Furthermore, Bhagat et al. reported that nicotine-induced acceleration of sinus rate was inhibited by hexamethonium but the DMPP-induced one was not suppressed by hexamethonium in isolated guineapig atria (6).

From the pharmacological point of view, it is of interest to investigate the chronotropic effects of nicotine and DMPP by utilization of blood-perfused SA node preparations of dogs. Previously Hashimoto and Chiba (7) demonstrated that tetrodotoxin was a selective blocking agent of nerve stimulation in the sinoatrial preparation and as such was a useful tool for excluding the effect of nerve excitation at peripheral organs. In this study, an attempt was made to analyse responses to nicotine and DMPP by use of tetrodotoxin in addition to autonomic drugs.

Preliminary data from this study has previously been reported (8).

MATERIALS AND METHODS

Twenty-one mongrel dogs of both sexes weighing from 10 to 15 kg were anesthetized with pentobarbital, 30 mg/kg i.v. Direct perfusion of the sinus node artery was performed under constant perfusion pressure at 100 mm Hg as reported previously (9, 10). The flow rate in the sinus node artery was recorded using a magnetic flowmeter (Nihon Kohden MF-2). Two electric manometers (Nihon kohden RP-2) were used to measure perfusion pressure of sinus node artery and systemic blood pressure at either the femoral or carotid artery. The heart rate was recorded using a cardiotachograph (Nihon Kohden RT-2) triggered by the R wave of lead II of ECG. The drug solution was injected at a volume of 0.01 to 0.05 ml in a period of 4 sec into the sinus node artery utilizing microinjectors.
Drugs used were nicotine (base), 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) (Aldrich), atropine sulfate, dl-norepinephrine hydrochloride (Sankyo), dl-propranolol (Sumimoto Chemicals), dl-(2-isopropylamino-1-hydroxyethyl) methanesulfonanilide hydrochloride (MJ1999) (sotalol) (Mead Johnson), hexamethonium bromide (Yamanouchi) and tetrodotoxin (TTX).

RESULTS

1) Effects of nicotine and DMPP on the SA node (Figs. 1, 2)

When nicotine or DMPP, at doses between 1 to 10 μg, was injected into the sinus node artery, a biphasic response, i.e., a negative chronotropic effect followed by a positive one, was usually observed as shown in Fig. 1. Doses above 30 μg resulted in considerable elevation in the systemic blood pressure (10 to 30 mm Hg). Tachyphylaxis did not develop.
when a second dose of the ganglion stimulants was given after responses to the first dose had completely disappeared. Fig. 2 shows that with 10 μg of nicotine no tachyphylaxis was seen during a 5 to 30 min interval. Response patterns to DMPP were similar to those of nicotine, but a following positive chronotropic effect of DMPP was usually greater than that of nicotine in the same dose range. Furthermore, large doses of the under the ganglion stimulants occasionally induced fibrillation. Injection of 10 μg of DMPP resulted in atrial fibrillation in 3 out of 15 dogs. In these instances a given dose of DMPP resulted in atrial fibrillation repetitively in the same animal. On the other hand, injection of nicotine into the sinus node artery did not induce atrial fibrillation at a dose of 10 μg in all experiments. Nicotine produced atrial fibrillation in 2 out of 8 cases at a dose of 30 μg.

2) Effects of atropine and adrenergic β-blocking agents, propranolol and sotalol (Fig. 3)

Negative responses to nicotine (10 μg) and DMPP (10 μg) were completely absent with atropine (1 μg). Positive response to either of these ganglion stimulants was completely blocked by an adrenergic β-blocking agent, propranolol or sotalol (MJ 1999). Fig. 3 shows that the negative chronotropic response to 10 μg of DMPP is blocked by 1 μg of atropine and the following positive one is blocked by 10 μg of sotalol (MJ 1999).

Atrial fibrillation induced by 10 μg of DMPP was also absent with 1 to 10 μg of atropine.

**Fig. 3.** Blocking effect of 1 μg of atropine on the negative chronotropic response to 10 μg of DMPP and blocking effect of 10 μg of MJ 1999 (sotalol) on the positive one. SBP, systemic blood pressure. HR, heart rate.

**Fig. 4.** Effect of 100 μg of hexamethonium (C₆) on nicotine- and DMPP-induced chronotropic responses.
(2 exps) and 1 μg of propranolol (3 exps).

3) Effect of hexamethonium (Fig. 4, Table 1)

Hexamethonium, 100 to 300 μg, injected into the sinus node artery induced a slight acceleration of sinus rate. The negative chronotropic responses to either of the ganglion stimulants (1 to 10 μg) were suppressed by treatment with hexamethonium (100 to 300 μg). Positive chronotropic response to nicotine was also blocked by hexamethonium. On the other hand, the DMPP-induced positive chronotropic response was not suppressed by hexamethonium. Fig. 4 shows that 100 μg of hexamethonium blocked the negative and positive chronotropic responses to 10 μg of nicotine and the negative chronotropic response to 1 μg of DMPP but did not block that of positive chronotropic to DMPP. These effects of hexamethonium disappeared 20 min later, however, the positive chronotropic response

| Drug (μg) | No. of dogs | Initial heart rate (beats/min) | Control | After 100 to 300 μg of C₆ |
|-----------|-------------|--------------------------------|---------|--------------------------|
|           |             |                                | DHR (%) | IHR (%)                  |
|           |             |                                | DHR (%) | IHR (%)                  |
|           |             |                                | DHR (%) | IHR (%)                  |
| Nicotine  | 10          | 5                              | 148±12  | 28±5                     |
|           |             |                                | 22±2    | 3±1                      |
|           |             |                                | 0       | 26±4                     |
|           |             |                                | 9±3     |                          |
| DMPP      | 1           | 3                              | 147±20  | 19±6                     |
|           |             |                                | 33±17   | 0                        |
|           |             |                                | 32±20   | 15±4                     |
|           |             |                                | 33±16   |                          |
|           | 3           | 3                              | 150±21  | 26±7                     |
|           |             |                                | 48±14   | 0                        |
|           |             |                                | 45±13   | 17±1                     |
|           |             |                                | 43±16   |                          |

C₆, hexamethonium.

DHR, decrease in heart rate. IHR, increase in heart rate.

Values are mean ± S.E.

Fig. 5. Effect of 1 μg of tetrodotoxin (TTX) on DMPP- and nicotine-induced chronotropic responses.
to nicotine was not restored to control level even after the complete recovery of DMPP action. Summarized data are shown in Table 1.

4) **Effect of tetrodotoxin** (Fig. 5, Table 2)

Tetrodotoxin at a dose of 1 µg injected into the sinus node artery produced a slight deceleration of the sinus rate. The deceleration response to nicotine and DMPP were abolished by use of tetrodotoxin. The acceleration response to nicotine was inhibited by treatment with tetrodotoxin. DMPP-induced positive chronotropic effect was not, however, blocked by tetrodotoxin. Fig. 5 shows that 1 µg of tetrodotoxin inhibited the responses to 10 µg of nicotine but did not block the positive chronotropic response to 1 µg of DMPP. Summarized data are shown in Table 2.

| Drug (µg) | No. of dogs | Initial heart rate (beats/min) | Control | After 1 µg of tetrodotoxin |
|-----------|-------------|--------------------------------|---------|--------------------------|
|           |             |                                | DHR (%) | IHR (%) | DHR (%) | IHR (%) | DHR (%) | IHR (%) |
| Nicotine  |             |                                |         |         |         |         |         |         |
| 1         | 3           | 152±9                          | 20±5    | 9±5     | 2±1     | 3±3     | 16±4    | 3±2     |
| 10        | 5           | 149±3                          | 29±4    | 14±2    | 1±1     | 2±2     | 19±4    | 9±3     |
| DMPP      |             |                                |         |         |         |         |         |         |
| 1         | 5           | 134±4                          | 23±5    | 24±6    | 3±1     | 23±7    | 21±5    | 23±6    |
| 3         | 3           | 140±12                         | 31±2    | 34±7    | 0       | 35±7    | 29±6    | 33±9    |

DHR, decrease in heart rate. IHR, increase in heart rate. Values are mean±S.E.

**DISCUSSION**

It is well known that the pacemaker cells are densely innervated with sympathetic and parasympathetic nerve fibers. Parasympathetic ganglia are found to be close around the SA node while sympathetic postsynaptic fibers end with terminals on the pacemaker cells (11). Nicotine induces a biphasic chronotropic effect so that the negative chronotropic response to nicotine is due to parasympathetic ganglionic excitation and the positive one is due to catecholamine released from terminals of sympathetic nerve fibers (6, 12).

As previously reported by Nadeau and James (5) treatment with atropine abolishes the initial deceleration while propranolol eliminates prolonged acceleration. The ability of DMPP to directly release catecholamines from the postganglionic sympathetic nerve endings has been suggested by Lindmar and Muscholl (13). Bhagat et al. (6) reported that DMPP-induced acceleration of sinus rate but was not inhibited by hexamethonium in the guinea-pig atrium preparations. In this study, it was demonstrated that the negative chronotropic responses to nicotine and DMPP were absent by pretreatment with atropine and the positive one by an adrenergic β-blocking agent, propranolol or sotalol. DMPP-induced acceleration of sinus rate was not, however, suppressed with either hexamethonium or tetrodotoxin,
although nicotine-induced acceleration was inhibited by both hexamethonium and tetrodotoxin. In 1969, Hashimoto and Chiba (7) demonstrated that tetrodotoxin had neither anticholinergic nor antiadrenergic properties, and did not block catecholamine release induced by tyramine, making the blocking effect of tetrodotoxin selectively limited to peripheral nerve excitation of the SA node area. It is therefore indicated that catecholamine release by nicotine is accompanied by excitation of sympathetic nerve fibers and this release by DMPP is produced without excitation of the fibers making the mechanism of a positive chronotropic response to DMPP different from that of nicotine.

A relatively large dose of DMPP above 10 μg or nicotine above 30 μg occasionally induced atrial fibrillation. Previously Hashimoto et al. (10) showed that atrial fibrillation was regularly induced by the administration of ACh into the sinus node artery. They also reported that the induction of atrial fibrillation was prevented by guanethidine, bretylium, DCI or propranolol and by pretreatment with reserpine. Furthermore, Hashimoto and Chiba (7) reported that tetrodotoxin prevented the induction of atrial fibrillation. Thus, it is suggested that the participation of adrenergic mechanism may play an important role in induction of atrial fibrillation by the administration of ACh into the sinus node artery. In the present study, a large amount of DMPP or nicotine caused the release of both ACh and norepinephrine. Thus, it is considered that atrial fibrillation probably results from the interaction between ACh and norepinephrine at the SA nodal region.

**SUMMARY**

The perfusion of the sinus node artery was performed on twenty-one dogs in situ. Nicotine at a dose of 1 to 10 μg and dimethylphenylpiperazinium (DMPP) at a dose of 1 to 3 μg injected into the sinus node artery induced a biphasic response, i.e., a negative chronotropic response followed by a positive one. A large dose of nicotine above 30 μg and DMPP above 10 μg occasionally induced atrial fibrillation. Both compounds produced similar negative chronotropic responses, but positive responses were more markedly induced by DMPP than nicotine at the same dose. These negative chronotropic responses were blocked by atropine, hexamethonium and tetrodotoxin. The positive response to nicotine was blocked by sotalol or propranolol, hexamethonium and tetrodotoxin. On the other hand, DMPP-induced positive chronotropic effect was blocked by a β-blocking agent, sotalol or propranolol but not by either hexamethonium or tetrodotoxin.

It is concluded that the positive chronotropic response to DMPP is different from that of nicotine with mechanism of action being similar to that to tyramine.

*Acknowledgments:* This study was supported by Pharmacological Research Foundation, Inc., and the Sankyo Central Laboratories. The authors express their sincere thanks to Mr. Keisuke Satoh, student of Tohoku University School of Medicine, for surgical assistance.
REFERENCES

1) McDowall, R.J.S.: J. Physiol. 104, 392 (1946)
2) Kotigoda, S.R.: Br. J. Pharmac. Chemother. 8, 83 (1953)
3) Ling, H.W.: Br. J. Pharmac. Chemother. 14, 505 (1959)
4) Chiang, T.S. and Leaders, F.E.: J. Pharmac. exp. Ther. 149, 225 (1965)
5) Nadeau, R.A. and James, T.N.: Am. J. Physiol. 212, 911 (1967)
6) Bhagat, B., Robinson, I.N., and West, W.L.: Br. J. Pharmac. Chemother. 30, 470 (1967)
7) Hashimoto, K. and Chiba, S.: J. Pharmac. exp. Ther. 170, 91 (1969)
8) Chiba, S., Satoh, S. and Hashimoto, K.: Tohoku J. exp. Med. 99, 407 (1969)
9) Hashimoto, K., Tanaka, M. and Chiba, S.: Circulation Res. 21, 297 (1967)
10) Hashimoto, K., Chiba, S., Tanaka, S., Hirata, M. and Suzuki, Y.: Am. J. Physiol. 215, 1183 (1968)
11) James, T.N.: Anat. Record 143, 251 (1962)
12) Burn, J.H. and Rand, M.J.: Br. med. J. 1, 137 (1958)
13) Lindmar, R. and Muscholl, E.E.: Arch. exp. Path. Pharmac. 242, 214 (1961)