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Effect of Tetramethylammonium on the Adrenal Medullary Secretion in the Dog

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In dogs anesthetized with Evipan-sodium, the adrenal venous blood was collected by the lumbar route method and was estimated chemically for adrenaline. Tetramethylammonium (TMA) injected in a dose of 1.0 mg per kg induced invariably the augmentation of adrenaline secretion of the adrenal gland with intact innervation as well as of the acutely denervated one. From the former the maximal secretion rate was measured on the average as 1.6 μg per kg per min. and from the latter as 1.1 μg. It is concluded that TMA induces an augmentation of adrenaline secretion chiefly by acting directly on the adrenal medulla. Adrenaline-secretory action of TMA was found to be about three times as strong as that of acetylcholine (ACh). Repeated TMA injections at five-minute intervals were made. The adrenaline secretion rate increased after each injection. Continuous infusion of TMA produced at first an increase in adrenaline secretion. During infusion the secretion rate decreased gradually.

It has been well established that TMA acts as a strong ganglionic stimulant. It has also been reported that TMA accelerates the adrenaline secretion of the adrenal gland.[2)3)4)]

The present investigation was undertaken to determine more completely the effects of TMA on adrenal medullary secretion.

EXPERIMENTAL METHODS

Eighteen dogs were used. The animals were anesthetized with Evipan-sodium. For collecting the adrenal venous blood, the lumbar route method of SATAKE *et al.*[5)] was applied. The adrenaline content of the sampled adrenal venous blood was estimated colorimetrically by the arseno-molybdic acid method of BLOOR and BULLEN.[1)] Adrenaline (Sankyo Co.) was used as the reference standard.

Intravenous administrations of chemicals were performed through a small cannula inserted into the saphenous vein. The duration of the TMA injection was 15 seconds except in cases of continuous TMA infusion. TMA salt used in the present investigation was tetramethylammonium bromide (Merck). In some experiments the effect of TMA on the adrenaline secretion was compared with that of ACh. In these experiments acetylcholine chloride (Hoffmann La Roche) was injected intravenously in 15 seconds.

The adrenal venous blood was collected before injection of TMA or ACh.

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Simultaneously with the onset of the injection, the collection of the adrenal venous blood was started again and performed successively during the first 60-second period, the second, the third, etc.

The denervation of the adrenal gland was made by cutting the splanchnic nerves in the corresponding side one or two hours before the start of observation.

RESULTS

Effect of tetramethylammonium on the adrenaline secretion rate of the adrenal gland with intact innervation. TMA was injected intravenously in a dose of 1.0 mg per kg of body weight in 15 seconds. The results obtained in four dogs are shown in Table 1.

In Exp. 1, the rate of adrenaline secretion before TMA injection was so small that it was immeasurable and it increased to 1.7 μg per kg per min. in the first 60-second period after the start of TMA injection. In the next 60-second period the rate of secretion decreased abruptly and was too small to be estimated.

TABLE 1.

Effect of Tetramethylammonium upon the Adrenaline Secretion Rate of the Adrenal Gland in Normal Dogs

No. of experiment	Body weight (kg) and sex	Adrenaline secretion rate (μg per kg per min.)					
		Before TMA injection	Minutes after TMA injection				
			0 – 1	1 – 2	2 – 3	3 – 4	4 – 5
1	8.2 ♂	*	1.7	*	*	*	*
2	11.0 ♂	0.01–0.02	1.6	0.14	0.02	0.01	0.01
3	10.8 ♀	0.02	1.4	0.01			
4	15.1 ♀	0.02	1.6	0.85	*	*	

* Immeasurably small amount of adrenaline in the adrenal venous blood.

The basal rate of adrenaline secretion in Exp. 2 was estimated to be 0.01–0.02 μg per kg per min. On receiving TMA it increased remarkably, 1.6 μg being reached in the first 60-second period. In the specimen taken during the second 60-second period it was estimated to be 0.14 μg. In the third 60-second period it resumed the pre-injection value.

In Exp. 3, the pre-injection rate of adrenaline secretion was 0.02 μg per kg per min. After injection of TMA, the secretion rate was increased and 1.4 μg were measured during the first 60-second period. In the second 60-second period it was found to resume the basal rate.

In Exp. 4, the adrenaline secretion rate before TMA was 0.02 μg per kg per min. It increased and reached 1.6 μg in the first 60-second period after the start of TMA injection. In the following 60-second period the augmented rate of secretion was still observed and it was 0.85 μg. In the third 60-second period it was immeasurably small.

Effect of tetramethylammonium on the adrenaline secretion rate of the denervated adrenal gland. Experiments were done in four dogs. TMA was applied intravenously in a dose of 1.0 mg per kg of body weight in 15 seconds. The results are summarized in Table 2.

TABLE 2.

Effect of Tetramethylammonium upon the Adrenaline Secretion Rate of the Adrenal Gland in Splanchnicotomized Dogs

No. of experiment	Body weight (kg) and sex	Adrenaline secretion rate (μg per kg per min.)			
		Before TMA injection	Minutes after TMA injection		
			0 – 1	1 – 2	2 – 3
5	16.7 ♀	*	0.53	0.12	0.06
6	20.6 ♀	0.03	1.1	0.13	0.01
7	13.5 ♂	0.05–0.07	1.6	0.05	0.02
8	13.3 ♂	0.02–0.03	1.3	0.06	0.05

* Immeasurably small amount of adrenaline in the adrenal venous blood.

In Exp. 5, the basal rate of adrenaline secretion was immeasurably small. On receiving TMA, the secretion rate increased in the first 60-second period after the start of injection, 0.53 μg per kg per min. being estimated. In the second 60-second period it was 0.12 μg.

In Exp. 6, the adrenaline secretion rate before the administration of TMA was 0.03 μg per kg per min. By the injection of TMA the secretion rate was increased, the highest being 1.1 μg, which was estimated in the first 60-second period after the start of injection. In the next 60-second period the secretion rate was 0.13 μg.

Similar results were obtained in Exp. 7. The pre-injection rate of adrenaline secretion was 0.05–0.07 μg per kg per min. It was accelerated by TMA injection to 1.6 μg in the first 60-second period after the start of injection, and in the second 60-second period it was measured as 0.05 μg.

In Exp. 8, the adrenaline secretion rate before TMA was 0.02–0.03 μg per kg per min. It increased markedly and was measured as 1.3 μg in the first 60-second period after the start of the application of TMA. And it was estimated to be 0.06 μg in the second 60-second period.

Comparative activities of tetramethylammonium and acetylcholine upon the adrenaline secretion rate of the denervated adrenal gland. Acetylcholine in doses of 1.0 mg, 2.0 mg, and 3.0 mg per kg was injected intravenously at intervals of half an hour. Before each ACh injection, atropine was given in a dose of 1.0 mg per kg in order to suppress the muscarine-like action of ACh. TMA was injected twice in a dose of 0.5 mg per kg with an interval of half an hour. The first TMA injection was made without previous administration of atropine and the second one was done after application of atropine in a dose of 1.0 mg per kg. The results obtained in three dogs are presented in Table 3.

TABLE 3.

Comparative Activities of Tetramethylammonium and Acetylcholine upon the Adrenaline Secretion Rate of the Adrenal Gland

No. of experiment	Body weight (kg) and sex	Dose of TMA or ACh (mg/kg)	Adrenaline secretion rate (μg per kg per min.)		
			Before injection	Minutes after injection	
				0 – 1	1 – 2
9	16.5 ♂	TMA 0.5	0.03	0.81	0.09
		TMA 0.5	0.04	0.63	0.08
		ACh 1.0	*	0.31	*
		ACh 2.0	0.02	1.1	*
		ACh 3.0	0.04	2.4	0.04
10	10.2 ♀	TMA 0.5	*	0.26	*
		TMA 0.5	*	0.27	0.01
		ACh 1.0	0.02	0.12	*
		ACh 2.0	0.01	0.39	0.02
		ACh 3.0	*	0.67	0.05
11	10.0 ♂	TMA 0.5	0.07	0.40	0.07
		TMA 0.5	0.04	0.24	0.06
		ACh 1.0	0.09	0.15	0.05
		ACh 2.0	0.04	0.44	0.04
		ACh 3.0	*	0.49	0.04

* Immeasurably small amount of adrenaline in the adrenal venous blood.

In Exp. 9, the adrenaline secretion rate before the first TMA injection was 0.03 μg per kg per min. By the application of TMA it was increased to 0.81 μg, which was estimated in the first 60-second period after the start of injection. In the second 60-second period it was 0.09 μg. Thirty minutes after the first TMA injection, atropine was applied. Then, TMA in the same dose as before was injected again. The rate of adrenaline secretion before the second TMA injection was 0.04 μg. On receiving TMA, it was accelerated to 0.63 μg during the first 60-second period. In the following 60-second period it was estimated as 0.08 μg. After atropinisation, ACh in a dose of 1.0 mg per kg was injected. Before ACh the adrenaline secretion rate was immeasurably small. By the injection of ACh it was increased and reached 0.31 μg in the first 60-second period. In the next 60-second period it was too small to be measured. Half an hour later, the administration of ACh in a dose of 2.0 mg was made after the application of atropine. The rate of secretion before ACh was 0.02 μg. After ACh, a maked increase in adrenaline secretion rate was observed, 1.1 μg being estimated in the first 60-second period. In the following 60-seconds period it was immeasurably small. Thirty minutes later, the injection of ACh in a dose of 3.0 mg per kg was done after atropine injection. The secretion rate of adrenaline before ACh was 0.04 μg. On receiving ACh, a marked increase in adrenaline secretion rate such as 2.4 μg was found in the first 60-second period. In the second 60-second period it was 0.04 μg.

Almost the same results were obtained in Exp. 10. On receiving TMA with and without previous administration of atropine, the rate of adrenaline secretion was increased and estimated at 0.26 μg and 0.27 μg per kg per min., respectively. ACh in doses of 1.0 mg, 2.0 mg, and 3.0 mg per kg was injected after application of atropine. Before each ACh injection the adrenaline secretion rate was 0.02 μg, 0.01 μg, and immeasurably small, respectively. After injection of ACh the secretion rate was accelerated, 0.12 μg, 0.39 μg, and 0.67 μg, being measured in the first 60-second period after the start of the injection, respectively. In the next 60-second periods the secretion rate was not more than 0.05 μg.

Similar results were obtained in Exp. 11. The rate of adrenaline secretion before the first TMA injection was 0.07 μg per kg per min. By the administration of TMA it increased and reached 0.40 μg in the first 60-second period. In the following 60-second period it resumed the pre-injection rate. Thirty minutes after the first TMA injection, the second TMA injection was made, which was preceded by the application of atropine. The adrenaline secretion rate

TABLE 4.

Effect of the Repeated Injections of Tetramethylammonium upon the Adrenaline Secretion Rate of the Adrenal Gland

No. of experiment	Body wkight (kg) and sex	No. of TMA injection	Adrenaline secretion rate (μg per kg per min.)		
			Before TMA injection	Minutes after TMA injection	
				0 – 1	1 – 2
12	12.1 ♂	1	*	0.87	*
		2	*	0.52	*
		3	*	0.69	*
		4	0.03	0.49	*
		5	*	0.71	*
13	11.3 ♀	1	*	0.20	*
		2	*	0.18	*
		3	*	0.40	*
		4	*	0.17	0.01
		5	0.01	0.13	*
14	13.8 ♂	1	0.06	1.2	0.13
		2	0.05	1.1	0.11
		3	*	1.2	0.14
		4	0.03	0.98	0.09
		5	0.03	0.48	0.07
15	14.4 ♀	1	*	2.6	0.24
		2	*	1.8	0.20
		3	*	1.5	0.19
		4		0.88	*
		5	0.17	0.95	0.25

* Immeasurably small amount of adrenaline in the adrenal venous blood.

before the TMA injection was 0.04 μg. After TMA it was measured as 0.24 μg in the first 60-second period. In the following 60-second period it was 0.06 μg. Then, after application of atropine, ACh in doses of 1.0 mg, 2.0 mg, and 3.0 mg was applied at intervals of 30 minutes. The adrenaline secretion rate before ACh was 0.09 μg, 0.04 μg, and immeasurably small, respectively. The rate after ACh was measured as 0.15 μg, 0.44 μg, and 0.49 μg, respectively. In the following 60-second period it was 0.05 μg, 0.04 μg, and 0.04 μg, respectively.

Effect of the repeated injections of tetramethylammonium upon the adrenaline secretion rate of the denervated adrenal gland. TMA was administered intravenously five times in a dose of 1.0 mg per kg of body weight at intervals of five minutes. The results obtained in four dogs are set out in Table 4.

In Exp. 12, the rate of adrenaline secretion before the first, the second, the third, and the fifth TMA injection was too small to be estimated. And that before the fourth injection was 0.03 μg per kg per min. After the start of injection it increased and was measured in the first 60-second periods after the first, the second, the third, the fourth, and the fifth injection as 0.87 μg, 0.52 μg, 0.69 μg, 0.49 μg, and 0.71 μg, respectively. In the next 60-second periods it was immeasurably small.

In Exp. 13, the pre-injection rate of adrenaline secretion was immeasurably small except before the fifth TMA injection, when it was measured as 0.01 μg per kg per min. On receiving TMA, it increased and was measured as 0.20 μg, 0.18 μg, 0.40 μg, 0.17 μg, and 0.13 μg in the first 60-second periods after the start of the first, the second, the third, the fourth, and the fifth injdction, respectively. In the next 60-second pericds it resumed the pre-injection value.

In Exp. 14, the adrenaline secretion rate before TMA injection was 0.06 μg, 0.05 μg, immeasurably small, 0.03 μg, and 0.03 μg, respectively. After TMA injection it was measured as 1.2 μg, 1.1 μg, 1.2 μg, 0.98 μg, and 0.48 μg in the first 60-second periods. In the following 60-second pericds it was 0.13 μg, 0.11 μg, 0.14 μg, 0.09 μg, and 0.07 μg.

Almost the same results were obtained in Exp. 15. The pre-injection secretion rate of adrenaline was too small to be estimated except before the last TMA injection, when it was measured as 0.17 μg per kg per min. After TMA injections, an enormous increase in adrenaline secretion was observed in the first 60-second periods, 2.6 μg, 1.8 μg, 1.5 μg, 0.88 μg, and 0.95 μg being measured. In the second 60-second periods the secretion rate of adrenaline decreased and was 0.24 μg, 0.20 μg, 0.19 μg, immeasurably small and 0.25 μg.

Effect of the continuous infusion of tetramethylammonium upon the adrenaline secretion rate of the denervated adrenal gland. TMA dissolved in saline solution was infused continuously at a speed of 2.0 mg per kg per min. into the saphenous vein. The colletion of the adrenal blood was started simultaneously with the onset of TMA infusion. Five collections were performed successively. Duration of each collection was 60-seconds. The results obtained in three dogs are summarized in Table 5.

In Exp. 16, the rate of adrenaline secretion before TMA was 0.09–0.10 μg per kg per min. On receiving TMA, the adrenaline secretion rate in the first 60-second period was markedly accelerated and estimated to be 3.3 μg. In the second 60-second period it promptly decreased and was measured as 0.94 μg. And then the rate of the adrenaline secretion decreased further and was 0.34 μg, 0.28 μg, and 0.35 μg in the third, the fourth, and the fifth 60-second period, respectively.

TABLE 5.

Effect of Continuous Infusion of Tetramethylammonium upon the Adrenaline Secretion Rate of the Adrenal Gland

No. of experiment	Body weight (kg) and sex	Adrenaline secretion rate (μg per kg per min.)					
		Before TMA infusion	Minutes after the start of TMA infusion				
			0 – 1	1 – 2	2 – 3	3 – 4	4 – 5
16	7.2 ♂	0.09–0.10	3.3	0.94	0.34	0.28	0.35
17	9.6 ♂	0.02	0.48	0.09	0.04	0.06	0.05
18	7.0 ♀	*	3.7	1.4	0.56	0.28	0.20

* Immeasurably small amount of adrenaline in the adrenal venous blood.

In Exp. 17, the rate of adrenaline secretion before infusion was 0.02 μg per kg per min. By the infusion of TMA the adrenaline secretion rate was increased and estimated to be 0.48 μg in the first 60-second period. And then it decreased, 0.09 μg, 0.04 μg, 0.06 μg, and 0.05 μg being measured in the respective 60-second periods.

Similar results were obtained in Exp. 18. The pre-injection rate of adrenaline secretion was immeasurably small. During the first 60-second period after the start of infusion, the enormously increased secretion rate of 3.7 μg per kg per min. was observed. In the second 60-second period it was measured as 1.4 μg. In the third, the fourth, and the fifth 60-second periods, it gradually diminished and was measured as 0.56 μg, 0.28 μg, and 0.20 μg, respectively.

In the above experiments, 3 to 5 minutes after the start of infusion dyspnea was observed. And 3 to 4 minutes later, the dogs died without exception.

DISCUSSION

The experimental results described above indicate that TMA acts to augment markedly the adrenaline secretion of the adrenal gland with intact innervation as well as that of the acutely denervated one.

In experiments with the single TMA injection the maximal adrenaline secretion rate was always observed in the first 60-second period after the start of TMA injection. It was measured as 1.6 (1.4–1.7) μg per kg per min. from the adrenal gland with intact innervation and 1.1 (0.53–1.6) μg from the acutely denervated one. Thus, TMA was proved to augment the adrenaline secretion chiefly by acting directly on the adrenal medulla, although the possibility that TMA acts partly through the central mechanism could not be excluded.

EICHHOLTZ [8] observed in cats and dogs under urethane anesthesia that TMA chloride, given in a dose of 10 mg, caused an increase in adrenaline secretion rate, which was measured as 2.8 μg per kg per min. in one dog, 0.22 μg, 0.7 μg, and 7 μg per kg per min. in cats. After splanchnic nerve section the adrenal venous blood was collected and estimated for adrenaline by means of the frog hind limb vessel preparation. His results in the dog were similar to those of the present investigation.

Using the method of suprareno-jugular anastomosis in dogs, HOUSSAY and MOLINELLI[4] demonstrated that TMA salts, when applied in large doses, incaeased the adrenaline secretion. Complete denervation of the adrenal gland did not suppress the increase in adrenaline secretion caused by TMA.

By measuring the comparative activities of TMA and ACh upon the adrenaline secretion of the adrenal gland, it was found that 0.5 mg TMA per kg was stronger than 1.0 mg ACh per kg and weaker than 2.0 mg ACh per kg. Thus, TMA seems to have a potency about three times as strong as ACh.

Repeated injections of TMA at intervals of five minutes were performed in four dogs. After each injection the adrenaline secretion increased markedly. In most cases the magnitude of increase diminished slightly in repeating TMA injections. This decrease in responsiveness of adrenal medulla to TMA was not so remarkable as that observed in experiments of repeated nicotine injections.[6]

Infusing TMA continuously, the adrenaline secretion rate increased and reached its maximum in the first 60-second period. Thereafter it decreased slowly. And the adrenaline secretion rate did not resume the pre-infusion level even at the end of the five-minute infusion period. Again, these results differed from those observed in nicotine-infusion experiments.[6] The paralyzing action of TMA on the adrenal medulla was found to be definitely weaker than that of nicotine.

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