Effect of Hexamethonium on the Augmented Adrenaline Secretion of the Adrenal Gland Causable by Insulin Hypoglycemia

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In view of the blocking effect of hexamethonium on the autonomic ganglia it is deemed desirable to ascertain whether the adrenal medulla is also paralysed by hexamethonium.

The previously reported work¹⁾ provided evidence that the acceleration of adrenaline secretion causable by acetylcholine was definitely suppressed by hexamethonium. Following up these acetylcholine experiments, a further attempt was made in the present investigation to know how hexamethonium affects the augmented adrenaline secretion causable by insulin hypoglycemia.

Some years ago, Schachter²⁾ demonstrated in dogs that an application of insulin followed by hexamethonium produced more intensive hypoglycemia than that without hexamethonium. From these results it was deduced by him that hexamethonium possessed the paralysing action on the adrenal medulla and suppressed the augmentation of adrenaline secretion causable by insulin hypoglycemia. The evidence presented by him is, however, an indirect one. In the present investigation experiments were made in estimating directly the rate of adrenaline secretion of the adrenal.

EXPERIMENTAL

Methods

Dogs (8.1–14.8 kg. in weight), anesthetized with evipan-sodium, were experimented on. For collecting the adrenal venous blood, the lumbar route preparation of Satake *et al.*³⁾ was applied. The blood pressure was recorded by a mercury manometer connected with the femoral artery. The blood sugar content was estimated by the method of Hagedorn and Jensen.

Five to 15 minutes before insulin injection, the initial adrenal blood specimens were collected. Then, insulin was injected intravenously in a dose of 7 units per kg. of body weight. Thirty, 60, 70, 80 and 88 minutes after insulin the adrenal venous blood was collected. Ninety minutes after insulin, hexamethonium (Bistrium bromide, Squibb) was given intravenously in a dose of 0.75 mg. per kg. Thereafter the adrenal blood was sampled at intervals. The adrenaline content of the sampled adrenal venous blood specimens was estimated colorimetrically by the Bloor & Bullen's arseno-molybdic acid method,⁴⁾ adrenaline (Sankyo Co.) being taken as the standard.

RESULTS AND DISCUSSION

The data are shown in table I.

As the control experiments, insulin alone was applied in 3 dogs without injecting hexamethonium.

In Exp. 1, the blood sugar content, which was 0.106–0.107% before insulin, decreased remarkably after insulin. It was determined as 0.072% 30 minutes after insulin application and 0.064% 60 minutes. Thereafter it decreased further and 150 minutes after insulin it reached 0.041%.

The initial adrenaline secretion rate in this dog was too small to be estimated. Thirty minutes after insulin injection the rate of adrenaline secretion was found to have increased to 0.15 μ g. per kg. per minute, 30 minutes later it increased further and 0.21 μ g. was reached. Then it was measured as 0.24 μ g. 70 minutes after insulin, 0.26 μ g. 80 minutes, 0.25 μ g. 88 minutes, 0.27 μ g. 100 minutes, 0.21 μ g. 110 minutes, 0.16 μ g. 120 minutes and 0.19 μ g. 150 minutes, respectively.

A similar result was obtained in Exp. 2.

The blood sugar content before insulin was 0.111%. It decreased markedly after insulin and was measured as 0.071% 30 minutes after insulin, 0.066% 60 minutes, 0.064 % 88 minutes, 0.066% 120 minutes and 0.072% 150 minutes.

The adrenaline secretion rate before insulin was immeasurably small. After insulin, the rate of secretion was found to increase and was determined as 0.05 μ g. per kg. per minute 30 minutes after injection, 0.07 μ g. 60 minutes, 0.09 μ g. 80 minutes, 0.10 μ g. 88 minutes, 0.12 μ g. 100, 110 and 120 minutes and 0.03 μ g. 150 minutes, respectively.

In Exp. 3, the blood sugar content decreased from 0.106–0.110% and 30 minutes after insulin it reached 0.061%. Thereafter it decreased gradually and was measured as 0.054% 60 minutes after insulin, 0.051% 88 minutes, and 0.049% 115 minutes, respectively.

The initial rate of adrenaline secretion in this dog was 0.04 μ g. per kg. per minute. It increased and reached 0.14 μ g. 30 minutes after in-

sulin injection. It was measured as 0.10 μ g. 60 minutes after insulin, 0.12 μ g. 70 minutes, 0.15 μ g. 80 minutes, 0.25 μ g. 88 minutes, 0.21 μ g. 100 minutes and 0.13 μ g. 115 minutes, respectively.

The hexamethonium-experiments were performed in 4 dogs.

In Exp. 4, the blood sugar content before insulin was 0.119–0.120%. It was reduced by insulin injection and 60–88 minutes after injection it was determined as 0.060–0.064%. Then hexamethonium was given. After hexamethonium blood sugar content increased only slightly.

The initial adrenaline secretion rate was in this experiment 0.02 μ g. per kg. per minute. After insulin it increased. It reached 0.13 μ g. 60 minutes after insulin and it was measured as 0.17 μ g., 0.38 μ g. and 0.30 μ g. 70, 80 and 88 minutes after insulin, respectively. Ninety minutes after insulin, hexamethonium was injected. Then the rate of adrenaline secretion was found to have decreased remarkably. It was estimated to be 0.08 μ g. 5 minutes after hexamethonium, 0.04 μ g. 10 minutes, 0.03 μ g. 20 minutes and 0.01 μ g. 60 minutes.

In Exp. 5, the blood sugar content before insulin was 0.115%. Thirty, 60 and 80 minutes after insulin it was determined as 0.063%, 0.058% and 0.054%, respectively. Then, hexamethonium was injected. The blood sugar content was gradually decreased.

Before insulin, the adrenaline secretion rate was 0.01 μ g. per kg. per minute. Thirty minutes after insulin it was increased and measured as 0.09 μ g. Sixty, 70, 80 and 88 minutes after insulin it was found to be 0.13 μ g., 0.28 μ g., 0.17 μ g. and 0.13 μ g., respectively. Hexamethonium was given 90 minutes after insulin. On receiving hexamethonium the rate of secretion was decreased markedly and was measured as 0.02 μ g. per kg. per minute 10 minutes after hexamethonium, immeasurably small 20 minutes and 0.006 μ g. 50 minutes, respectively.

In Exp. 6, the blood sugar content was reduced from 0.123–0.124% to 0.060% within 30 minutes after insulin. From 60 to 80 minutes after insulin it was 0.054%. Then the injection of hexamethonium was made. The blood sugar decreased further and reached 0.048% 140 minutes after insulin.

The basal rate of adrenaline secretion in this experiment was 0.05–0.06 μ g. per kg. per minute. After insulin the rate of secretion was found to increase and was determined to be 0.23 μ g. per kg. per minute 30 minutes, 0.21 μ g. 60 minutes, 0.23 μ g. 70 minutes, 0.22 μ g. 80 minutes and 0.25 μ g. 88 minutes after insulin. Ninety-five minutes after insulin application, hexamethonium was injected. The rate of secretion then decreased remarkably and it was 0.03 μ g. per kg. per minute 10 minutes after hexamethonium, 0.02 μ g. 30 and 45 minutes.

In Exp. 7, the blood sugar content decreased from 0.112–0.114% to

TABLE I
The Adrenaline Secretion Rate after Insulin
with and without Hexamethonium

Time before or after injection of insulin (min.)	No. of specimen	Quantity of blood (cc.)	Duration of collection (sec.)	Blood flow per kg. per minute (cc.)	Adrenaline content in 1 cc. blood (μ g.)	Adrenaline secretion rate per kg. per minute (μ g.)	Blood sugar (%)	Blood pressure (mm Hg.)	Heart rate (per min.)	Respiration (per min.)	Body temperature ($^{\circ}$ C)	Room temperature ($^{\circ}$ C)
(A) Insulin												
Exp. 1. Dog 14.8 kg. ♀												
before												
15	1	3.8	30	0.51	*		0.107	140	160	23	39.0	14
5	2	4.0	30	0.54	*		0.106	136	136	25	38.9	14
Intravenous injection of insulin (7 units per kg.)												
after												
30	3	6.4	30	0.87	0.17	0.15	0.072	132	156	37	39.0	14.5
60	4	4.1	20	0.83	0.25	0.21	0.064	114	160	40	39.0	14.5
70	5	3.0	20	0.61	0.39	0.24	0.059	106	166	43	39.1	14.5
80	6	3.0	20	0.61	0.42	0.26	0.055	128	170	36	39.1	14.5
88	7	3.1	20	0.63	0.39	0.25	0.054	106	175	40	39.1	14.5
100	8	3.4	20	0.69	0.38	0.27	0.047	110	194	36	38.9	14.5
110	9	3.4	20	0.69	0.30	0.21	0.045	112	198	36	38.9	14.5
120	10	3.4	20	0.69	0.23	0.16	0.045	110	200	36	38.7	14.5
150	11	3.3	20	0.67	0.28	0.19	0.041	106	165	22	38.8	14.5
Exp. 2. Dog 13.3 kg. ♂												
before												
15	1	1.7	30	0.26	*		0.111	138	130	52	38.8	19
5	2	1.7	30	0.26	*		0.111	136	148	58	38.8	19
Intravenous injection of insulin (7 units per kg.)												
after												
30	3	1.9	30	0.29	0.18	0.05	0.071	122	150	70	38.1	19
60	4	1.7	30	0.26	0.27	0.07	0.066	122	146	66	37.9	19
70	5	1.3	30	0.20	0.30	0.06	0.064	116	148	68	37.8	19
80	6	2.1	30	0.32	0.28	0.09	0.064	118	150	52	37.4	19
88	7	2.2	30	0.33	0.31	0.10	0.064	108	142	62	37.5	19
100	8	1.8	30	0.27	0.43	0.12		106	146	56	37.4	19
110	9	1.8	30	0.27	0.46	0.12	0.067	108	150	58	37.5	19
120	10	2.0	30	0.30	0.41	0.12	0.066	104	148	52	37.5	19
150	11	1.6	30	0.24	0.14	0.03	0.072	110	158	48	37.4	19
Exp. 3. Dog 8.1 kg. ♀												
before												
15	1	2.0	30	0.49	0.08	0.04	0.106	116	170	20	38.3	15.5
5	2	1.8	30	0.44	0.09	0.04	0.110	122	158	22	38.1	15.5
Intravenous injection of insulin (7 units per kg.)												
after												
30	3	1.6	30	0.40	0.36	0.14	0.061	110	135	23	37.8	15.5
60	4	2.5	60	0.31	0.33	0.10	0.054	102	132	20	37.8	15.5
70	5	2.2	60	0.27	0.43	0.12	0.053	104	148	22	37.2	15.5
80	6	1.4	30	0.35	0.42	0.15	0.051	104	165	21	37.1	15.5
88	7	1.3	30	0.32	0.77	0.25	0.051	100	160	25	37.0	15.5
100	8	1.3	30	0.32	0.67	0.21	0.049	102	165	24	36.9	15.5
115	9	1.7	60	0.21	0.61	0.13	0.049	98	164	18	36.9	15.5

(B) Insulin-Hexamethonium**Exp. 4. Dog 9.4 kg. ♂**

before

10	1	1.8	30	0.37	0.06	0.02	0.120	154	220	26	37.8	12.5
5	2	1.7	30	0.35	0.06	0.02	0.119	152	185	20	37.7	12.5

Intravenous injection of insulin (7 units per kg.)

after

30	3	1.8	30	0.37	0.24	0.09	0.112	140	203	26	37.5	12
60	4	1.9	30	0.41	0.31	0.13	0.064	132	215	23	37.3	12
70	5	2.0	30	0.43	0.40	0.17	0.060	132	215	25	37.5	12
80	6	2.5	30	0.53	0.72	0.38	0.062	126	200	18	37.5	12
88	7	2.7	30	0.57	0.52	0.30	0.064	130	205	22	37.8	12
90	Intravenous injection of hexamethonium (0.75 mg. per kg.)											
95	8	1.7	30	0.35	0.22	0.08	0.068	90	144	18	37.2	11.5
100	9	1.5	30	0.33	0.11	0.04		92	134	16	36.8	11.5
110	10	1.3	30	0.28	0.09	0.03		78	133	20	36.9	11.5
120	11	1.8	30	0.37	0.13	0.04	0.071	76	138	15	36.5	11.5
150	12	1.7	30	0.35	0.03	0.01	0.075	80	140	17	35.9	11.5

Exp. 5. Dog 9.5 kg. ♂

before

15	1	1.4	30	0.29	0.05	0.01	0.115	134	145	33	37.6	12.5
5	2	1.4	30	0.29	*		0.115	134	145	35	37.6	12.5

Intravenous injection of insulin (7 units per kg.)

after

30	3	1.7	30	0.36	0.26	0.09	0.063	134	122	56	37.1	12.5
60	4	1.8	30	0.38	0.33	0.13	0.058	128	135	68	37.2	12.5
70	5	1.9	30	0.40	0.71	0.28		132	126	60	37.2	12.5
80	6	1.7	30	0.36	0.47	0.17	0.054	104	133	72	37.1	12.5
88	7	2.0	30	0.42	0.32	0.13		102	135	60		
90	Intravenous injection of hexamethonium (0.75 mg. per kg.)											
100	8	2.0	60	0.21	0.11	0.02	0.051	74	86	40	37.0	12
110	9	1.7	60	0.18	*		0.041	64	80	24	36.8	12
140	10	1.0	90	0.07	0.08	0.006	0.039	94	111	7	35.6	12

Exp. 6. Dog 10.1 kg. ♂

before

15	1	2.3	30	0.46	0.11	0.05	0.123	140	185	37	38.9	12
5	2	2.2	30	0.44	0.13	0.06	0.124	140	192	40	38.8	12

Intravenous injection of insulin (7 units per kg.)

after

30	3	2.1	30	0.42	0.54	0.23	0.060	124	132	59	39.0	12
60	4	1.8	30	0.36	0.59	0.21	0.054	132	160	61	38.9	12
70	5	1.8	30	0.36	0.64	0.23	0.054	124	175	66	38.8	11.5
80	6	1.5	30	0.30	0.74	0.22	0.054	118	165	60	38.8	11.5
88	7	1.6	30	0.32	0.79	0.25	0.053	120	148	42		
95	Intravenous injection of hexamethonium (0.75 mg. per kg.)											
105	8	1.4	60	0.14	0.24	0.03	0.047	88	130	33	38.5	12
125	9	1.8	60	0.18	0.13	0.02	0.047	104	136	40	37.7	12
140	10	1.5	60	0.15	0.16	0.02	0.048	100	135	26	37.4	12

Exp. 7. Dog 14.0 kg. ♂

before

15	1	1.8	60	0.13	0.16	0.02	0.112	146	165	14	38.5	14.5
5	2	1.9	60	0.14	0.15	0.02	0.114	120	188	16	38.2	14.5

Intravenous injection of insulin (7 units per kg.)

after

30	3	1.5	60	0.11	0.31	0.03	0.066	112	180	20	38.1	14
60	4	1.2	60	0.09	0.66	0.06	0.051	94	163	27	38.0	14
70	5	1.2	60	0.09	0.60	0.05	0.047	94	170	29	37.9	14
80	6	1.8	60	0.13	0.64	0.08	0.045	98	175	28	37.8	14
88	7	1.4	60	0.10	0.68	0.07		94	165	30		14
90	Intravenous injection of hexamethonium (0.75 mg. per kg.)											
100	8	1.5	60	0.11	0.12	0.01	0.039	70	121	20	37.1	14
110	9	1.8	90	0.09	0.22	0.02	0.035	72	115	20	36.9	14
120	10	1.3	60	0.09	0.22	0.02	0.035	74	120	20	36.7	14
150	11	1.9	60	0.14	0.15	0.02	0.030	82	128	8	36.1	14

* Less than 0.05 μ g.

0.066% 30 minutes after insulin and it was 0.045% 80 minutes, 0.039% 100 minutes, 0.035% 120 minutes and 0.030% 150 minutes after insulin.

In this experiment, the initial adrenaline secretion rate was 0.02 μ g. per kg. per minute. On receiving insulin, the rate of secretion was measured as 0.03 μ g. 30 minutes after insulin, 0.06 μ g. 60 minutes, 0.05 μ g. 70 minutes, 0.08 μ g. 80 minutes and 0.07 μ g. 88 minutes, respectively. Hexamethonium was then injected. The secretion rate of adrenaline was decreased and was measured as 0.01 μ g. per kg. per minute 10 minutes after hexamethonium and 0.02 μ g. 20, 30 and 60 minutes.

Comparing the results obtained in the hexamethonium-experiments mentioned above with those of the control ones, it is evident that the augmented adrenaline secretion causable by insulin hypoglycemia is suppressed by the application of hexamethonium.

In my previous investigation it was proved that the augmentation of adrenaline secretion causable by acetylcholine was inhibited by hexamethonium. Since acetylcholine acts exclusively peripherally on the adrenal medulla to accelerate the adrenaline secretion, it is sure that in this case the site of action of hexamethonium is the adrenal medulla itself.

Therefore it is reasonable to suppose that in the case of insulin hypoglycemia, which acts through the central mechanism to augment the adrenaline secretion in releasing acetylcholine at the terminals of adrenalinosecretory nerve fibres, hexamethonium suppresses the augmentation of adrenaline secretion also by acting directly on the adrenal medulla.

SUMMARY

In dogs anesthetized with evipan-sodium, the adrenal venous blood specimens were collected through the lumbar route preparation. The adrenaline content of the specimens was measured colorimetrically by means of the Bloor & Bullen's arseno-molybdic acid method. The blood sugar content was also determined simultaneously.

Insulin was applied intravenously in a dose of 7 units per kg. of body weight. The adrenaline secretion rate was increased gradually, and from 1 to 2 hours after insulin a definite increase was invariably observed. At the height of augmentation of adrenaline secretion, hexamethonium was injected intravenously in a dose of 0.75 mg. per kg. In all cases the secretion rates were cut down to low levels by hexamethonium. It was measured in 3 cases as 0.02–0.04 μ g. per kg. per minute after hexamethonium against 0.13–0.30 μ g. before hexamethonium. In one case, it was 0.01 μ g. against 0.07 μ g.

Thus it is concluded that hexamethonium acts to inhibit the accelerating adrenaline secretion causable by insulin hypoglycemia.

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References

- 1) Yamashita, K., Jap. J. Physiol., 1956, **6**, 279.
 - 2) Schachter, M., J. Physiol., 1951, **115**, 206.
 - 3) Satake, Y., Sugawara, T. & Watanabe, M., Tohoku J. Exp. Med., 1927, **8**, 501.
 - 4) Bloor, W. R. & Bullen, S. S., J. Biol. Chem., 1941, **138**, 727.
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