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RESERPINE, MONOAMINE OXIDASE INHIBITORS, AND DISTRIBUTION OF BIOGENIC AMINES IN MONKEY BRAIN

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Abstract—Twenty-one macacus rhesus monkeys were given reserpine, and monoamine oxidase inhibitors (nialamide and isocarboxazid), both singly and in combination, and the levels of serotonin and norepinephrine were determined in various brain regions. The distributional pattern of these amines in monkey brain followed closely that described for other mammals. This primate species resembles rodents, not cats or dogs, in its biochemical response to monoamine oxidase inhibitors inasmuch as nialamide and isocarboxazid increased the brain levels not only of serotonin but also that of norepinephrine. The two inhibitors differed considerably in their interaction with reserpine. The combination of reserpine and isocarboxazid resulted in lower levels of brain amines than when the inhibitor was given alone. The combination of reserpine and nialamide resulted in higher brain serotonin levels than when only nialamide was administered. For this reason it is concluded that, under our experimental conditions, nialamide exerted more than the single action of inhibition of monoamine oxidase.

THE distribution of biogenic amines in the central nervous system is known for several species[1–5] including man.[6–8] In addition the effects of reserpine and many monoamine oxidase inhibitors have been extensively studied in rodents (mice,[9, 10] rats,[10, 11] guinea pigs,[10] and rabbits[10, 12]) and to a limited extent in cats[12–14] and dogs.[12, 15] Because comparable data for primates are lacking we initiated a study of the distribution of norepinephrine and serotonin in the brain of the macacus rhesus monkey. We also studied the effect of reserpine and the monoamine oxidase inhibitors, nialamide and isocarboxazid, given both singly and in combination on the levels of these amines in the brain.

METHODS

Twenty-one macacus rhesus, weighing between 3·3 and 8·0 kg were used for this study.* Five monkeys served as controls and groups of two or three animals received the following drugs:† nialamide, 25 mg/kg; isocarboxazid, 2 or 20 mg/kg; reserpine, 1 mg/kg; reserpine 1 mg/kg and nialamide 25 mg/kg; reserpine 1 mg/kg and isocarboxazid either 2 or 20 mg/kg. All drugs were injected intramuscularly once a day for 6 days and the animals sacrificed on the seventh day. The animals were anesthetized

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with pentobarbital, the carotid arteries cannulated and the head perfused with 300 to 500 ml of saline prior to removing the brain. The brain was rapidly dissected and sections frozen on solid carbon dioxide for subsequent analysis. The following regions were studied: pons-medulla, midbrain-hypothalamus, thalamus (mainly midline nuclei), caudate nucleus, hippocampus-amygdala, temporal pole, various cortical regions, and a portion referred to as "lenticular thalamic mass" consisting of the lateral thalamic nuclei, the internal capsule, putamen, and globus pallidus. Norepinephrine and serotonin were determined by the method of Shore and Olin[16] as modified by Mead and Finger.[17] When sufficient material was available duplicate determinations were performed and internal standards used.

RESULTS

In the brains of the control animals both serotonin (Table 1 and Fig. 1) and norepinephrine (Table 2 and Fig. 1) followed the pattern of distribution described for other

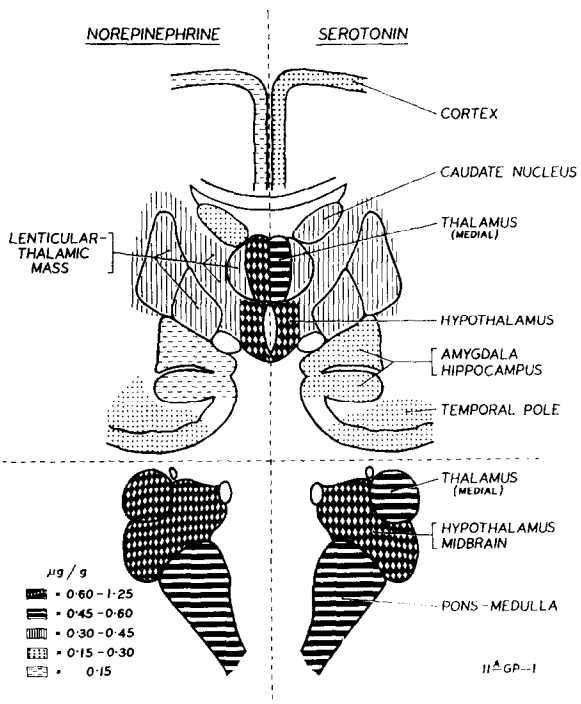

FIG. 1. Distribution of norepinephrine and serotonin in monkey brain.

mammals.[1–8] The highest amounts were found in the midbrain-hypothalamus, thalamus, and pons-medulla, in that order. Other regions contained appreciably less of either amine. Giarman *et al.*,[18] however, found that the pineal gland of both man and rhesus monkeys contains large amounts of serotonin, up to 22·8 and 10·0 μg/g respectively. None of the brain regions analyzed in this study approached these high values.

TABLE 1. SEROTONIN CONTENT OF MONKEY BRAIN REGIONS AFTER RESERPINE AND MONOAMINE OXIDASE INHIBITORS

	Control	Nialamide (25 mg/kg)	Isocarboxazid (2 mg/kg)	Isocarboxazid (20 mg/kg)	Reserpine (1 mg/kg)	Reserpine (1 mg/kg) + nialamide (25 mg/kg)	Reserpine (1 mg/kg) + isocarboxazid (2 mg/kg)	Reserpine (1 mg/kg) + isocarboxazid (20 mg/kg)
No. of animals	5	3	2	2	2	3	2	2
		136	82	199	14	198	38	118
Pons-medulla	49±11*	161	68	166	18	130	38	137
	(5)	91				86		
		123	118	323	24	330	52	166
Midbrain=hypothalamus	77±16	206	94	216	31	154	75	255
	(5)	137				127		
		65	69	137	24	230	27	78
Caudate	36±6	129	48	148	19	102	33	163
	(5)	53				102		
		76	62	332	16	229	75	277
Thalamus	56±15	149	111	172	115	143	74	209
	(5)	108				109		
		56	78	71	8	71	35	48
Hippocampus=amygdala	29±8	55	34	76	12	63	44	70
	(5)	38				50		
		(24–60)	15	42	(3–21)		11	39
Various cortical structures	17±4	41	17	38	9		16	35
	(8)	(7)			(6)			
Temporal pole	20	57	44	85		77	18	63
	24	42	35	77		46	20	62
						44		
Lenticular thalamic mass	29	48	41	110	8	141	26	78
	35		42	105	15	85	30	102
						66		

* Entries represent $\mu g \times 100$ of amine l/g of fresh brain. For controls the average, standard deviation, and number of determinations (in parentheses) are given. Other entries are individual values unless more than three determinations were made in which case the average and range are given.

TABLE 2. NOREPINEPHRINE CONTENT OF MONKEY BRAIN REGIONS AFTER RESERPINE AND MONOAMINE OXIDASE INHIBITORS

	Control	Nialamide (25 mg/kg)	Isocarboxazid (2 mg/kg)	Isocarboxazid (20 mg/kg)	Reserpine (1 mg/kg)	Reserpine (1 mg/kg) + nialamide (25 mg/kg)	Reserpine (1 mg/kg) + isocarboxazid (2 mg/kg)	Reserpine (1 mg/kg) + isocarboxazid (20 mg/kg)
No. of animals	4	3	2	2	2	3	2	2
		104	126	152	5	49	35	46
Pons=medulla	52±9*	118	74	128	4	20	40	58
	(4)	132				47		
		216	250	348	6	154	47	100
Midbrain=hypothalamus	125±16	295	156	144	8	58	45	139
	(4)	151				64		
		36	75	90	9	63	40	42
Caudate	27±10	41	49	114	10	38	50	40
	(4)	63				53		
		103	120	528	8		35	105
Thalamus	100±34	247	250	250	7		57	145
	(4)	116				95		
	(0–34)	8	51	60	3		19	19
Hippocampus=amygdala	11±16	16	19	44	2		33	19
	(4)	45				63		
	(0–24)	(15–46)	25	68	(0–7)	(3–31)	9	41
Various cortical structures	13±12	33	14	52	3	17	15	28
	(8)	(5)			(6)	(6)		
			16	46		17	14	23
Temporal pole	18	13	20	42		9	20	17
	(1)	32				23		
			25	93	2	25	18	38
Lenticular thalamic mass	38		45	80	0	21	22	44
	(1)	48				30		

* See footnote to Table 1.

As expected, continued administration of reserpine reduced the brain levels of both amines to low values in all areas except for one inexplicably high norepinephrine value in the thalamus. Both nialamide and isocarboxazid elevated brain levels of serotonin and norepinephrine in nearly all brain regions. Nialamide (25 mg/kg) was more effective than 2 mg of isocarboxazid per kg but less potent than 20 mg of isocarboxazid per kg.

Simultaneous administration of both reserpine and isocarboxazid for 6 days yielded levels of brain amines intermediate between those observed when the drugs were given separately. Dosage was important; isocarboxazid at 20 mg/kg was more effective in counteracting the reserpine-induced depletion of brain amines than at 2 mg/kg. These psychoactive drugs therefore have antagonistic biochemical actions in the monkey as in other species where they shift biogenic amine levels in opposite directions.[11, 14, 19, 20] If the effects of the drug combinations are compared with those of the inhibitors alone it is seen that the reserpine-induced depletion of serotonin was counteracted to a greater extent than that of norepinephrine. Norepinephrine levels observed after the combination of reserpine 1 mg/kg and isocarboxazid 20 mg/kg were less than those after isocarboxazid alone at 2 mg/kg. On the other hand, serotonin levels after the same combination were only slightly less than those observed after isocarboxazid at 20 mg/kg. The other drug combinations (reserpine 1 mg/kg and nialamide 25 mg/kg; reserpine 1 mg/kg and isocarboxazid 2 mg/kg) produced similar changes for norepinephrine. Comparable observations have been made in rodents, although in most experiments one of the drugs was injected before the other. One surprising exception to this generalization was encountered. With the combination of reserpine 1 mg/kg and nialamide 25 mg/kg higher serotonin levels were observed in some brain regions (caudate nucleus, thalamus, midbrain, hypothalamus), than with 25 mg of nialamide per kg alone.

The gross behavior of the monkeys agreed in general with the biochemical data and known pharmacological action of the drugs. Except for one of the monkeys receiving isocarboxazid (20 mg/kg), which became excitable and exhibited twitching, no behavioral changes were noted in the animals given only isocarboxazid or nialamide. When reserpine was administered the animals became progressively more docile as the injections proceeded. During the last 3 days of the experiment they were easily removed from their cages without the usual safety precautions and they expressed little interest in events occurring in the animal room. In contrast, none of the monkeys receiving reserpine and a monoamine oxidase inhibitor could be readily captured for injection nor did they lose their normal curiosity.

DISCUSSION

All monoamine oxidase inhibitors investigated previously elevated both norepinephrine and serotonin levels in the brains of rodents,[10–13, 19–21] but no monoamine oxidase inhibitor is known that will elevate brain norepinephrine levels in the cat even though high serotonin levels may be achieved.[13] In the dog only nialamide was effective in elevating both amines in the brain.[12, 15] The ability of isocarboxazid to increase norepinephrine levels in the rhesus macacus indicates that primates resemble rodents, not cats or dogs, in their biochemical response to monoamine oxidase inhibitors. Schneider *et al.*[22] gave nialamide (50 mg/kg per day for 35 days) to a monkey and observed a twofold increase of both brain amines.

The ability of reserpine to deplete brain biogenic amines is well known but its mechanism of action is still under discussion. It is not clear in what form the residual amines exist in the brain or how monoamine oxidase inhibitors interact with reserpine. Green and Erickson[19] have suggested that the residual fluorescence measured in brain extracts after appropriate doses of reserpine may not represent actual serotonin but rather a nonspecific "tissue blank". The fluorescence spectra of many of our extracts obtained from monkeys receiving reserpine agree with those presented by Green and Erickson in that a relatively large portion of the fluorescence at 550 mμ was due to nonspecific fluorescence resembling the reagent blank. However, extracts of areas normally rich in serotonin did show a small, broad peak in this region. Therefore, until this point is resolved we wish to regard our data as representing apparent serotonin content. In any event, the absolute contribution of nonspecific fluorescence is only a small fraction of the amount normally present.

Surprisingly, reserpine plus nialamide produced a higher serotonin level in brain than nialamide by itself. This agrees with the recent finding of Green and Erickson[19] for tranylcypromine. They reported that, even though reserpine caused a reduction of norepinephrine to control levels when given to rats several hours after that monoamine oxidase inhibitor, it also produced a large increase of serotonin over that evoked by tranylcypromine alone. Reserpine combined with iproniazid did not act similarly in their experiments nor did reserpine combined with isocarboxazid do so in ours. However, Funderburk *et al.*[13] reported that reserpine given after nialamide to rats produced levels of serotonin lower than those with nialamide alone. Similar observations have been made in our laboratory. These experiments demonstrate that monoamine oxidase inhibitors differ considerably from one another in their interaction with reserpine and that under our conditions the sole action of nialamide in the monkey cannot be simply inhibition of monoamine oxidase.

Evidence indicating that monoamine oxidase inhibitors do not block the reserpine-induced release of amines from their binding sites was presented by Spector *et al.*[11] According to their view the principal action of these inhibitors even in the presence of of reserpine is to prevent the destruction of the amines by monoamine oxidase. However, Schanberg and Giarman[23] feel that iproniazid interacts with reserpine at the binding sites of serotonin. Green and Erickson[19] on the other hand postulate an additional mechanism for interaction of these compounds, suggesting that reserpine suppresses the formation of serotonin and that certain monoamine oxidase inhibitors may reverse this process, a hypothesis which may apply to nialamide. Our experiments do not bear directly on the question of the mechanism of antagonism between reserpine and monoamine oxidase inhibitors but they do show that brain levels of both serotonin and norepinephrine can be maintained above control values by the simultaneous administration of a monoamine oxidase inhibitor to monkeys receiving sufficient reserpine to cause marked depletion of the biogenic amines. It is also becoming clear that many factors affect the interaction of reserpine and monoamine oxidase inhibitors, among which are relative doses of each drug, time of pretreatment, nature of the monoamine oxidase inhibitor, and the particular species under investigation.

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