Nicotine is accumulated intracellularly in ganglia (Brown, Halliwell & Scholfield, 1969). This might form a slowly clearing reservoir sustaining extracellular nicotine levels and consequent receptor activation during washout.

A pertinent question arising from this work is whether similar explanations, rather than postulation of separate hyperpolarizing receptors, might account for some other instances of increased ganglionic hyperpolarization following nicotinic blocking agents.

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The effect of drug pretreatment on synaptic activity in *Helix* brain

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The effect of pretreatment with a series of compounds on two inhibitory dopamine pathways, one excitatory cholinergic pathway and one excitatory 5-hydroxytryptamine (5-HT) pathway in the snail brain has been investigated. One dopamine pathway produced an inhibitory postsynaptic potential (ipsp) while the other produced an inhibition of long duration (ILD) lasting for several seconds (Kerkut, Horn & Walker, 1969). Both excitatory pathways produced excitatory postsynaptic potentials (epsps) following presynaptic stimulation.

Compounds were injected into the snail haemocoel in 0·2 ml distilled water. Stimuli were applied to the relevant nerve at a frequency of 1·2 Hz. The voltage was selected to give a unitary monosynaptic response. The size of the potential after one stimulus is termed the initial height. Following repetitive stimulation the epsp declined while the ipsp increased to a constant height, the final height. The drug effect on initial and final heights and on the number of stimuli required to give the final height are recorded in Table 1.

Para-chlorophenylalanine, α-methyl-5-hydroxytryptophan or reserpine reduced the initial and final heights of the 5-HT epsp. *Para*-chlorophenylalanine also decreased the number of stimuli required to give the final height. Hemicholinium reduced the initial and final heights of the cholinergic epsp. The time taken for the epsp amplitude to fall to a constant amplitude was also reduced.

Alpha-methyl-3-4-dihydroxyphenylalanine, α-methyl-*p*-tyrosine or 6-hydroxydopamine reduced the initial and final heights of the ipsp and the ILD height. α-Methyl-3-4-dihydroxyphenylalanine and α-methyl-*p*-tyrosine also reduced the final height

TABLE 1. *Effect of pretreatment with a series of compounds on synaptic potentials from one cholinergic, one 5-HT and two dopamine pathways in the snail brain*

Drug	5-HT epsp			Acetylcholine epsp			Dopamine ipsp			Dopamine ILD	
	Initial height mV	Final height mV	No. of stimuli	Initial height mV	Final height mV	No. of stimuli	Initial height mV	Final height mV	No. of stimuli	Height mV	Length sec
Control Mean	7·81	3·69	20·2	5·97	2·78	24·9	4·28	6·66	7·98	1·47	2·65
±S.E.M.	0·44	0·21	1·37	0·32	0·22	1·83	0·18	0·20	0·62	0·07	0·23
p-Chlorophenylalanine	4·02	1·69	14·6	6·0	2·71	24·9	2·50	2·19	11·0	1·16	1·17
	0·82	0·45	1·34	0·33	0·22	1·1	0·71	0·90	1·98	0·37	0·47
P	<0·001	<0·001	<0·05								
Hemicholinium	6·88	3·13	22·8	3·93	1·55	15·3	4·06	6·88	6·33	2·13	3·6
	0·46	0·16	1·64	0·35	0·20	1·1	0·37	0·43	0·49	0·62	0·92
				<0·01	<0·01	<0·01					
L-3-4-dihydroxyphenylalanine	7·23	3·21	22·7	7·11	3·17	26·6	8·8	11·76	7·0	2·11	2·65
	0·49	0·45	1·52	0·61	0·24	1·83	0·75	1·03	0·73	0·18	0·23
							<0·001	<0·001		<0·001	
α-Methyl-3-4-dixhydroxyphenyl-alanine	6·34	2·86	22·3	6·77	3·26	25·2	2·58	4·12	7·33	1·43	1·43
	0·40	0·17	1·7	<0·45	0·31	1·34	0·53	0·72	1·15	0·30	0·29
		<0·05					<0·05	<0·01			<0·01
5-Hydroxytryptophan	9·06	4·38	28·3	7·14	3·21	23·7	3·76	5·82	4·86	1·82	2·23
	0·46	0·35	1·63	0·38	0·21	1·55	0·61	0·91	0·51	0·69	0·44
			<0·01								
α-Methyl-5-hydroxytryptophan	5·09	2·23	18·6	6·96	3·21	25·3	3·44	5·96	5·33	2·04	1·19
	0·44	0·30	1·32	0·40	0·32	1·16	0·39	0·68	0·49	0·71	0·31
	<0·01	<0·01							<0·05		
α-Methyl-*p*-tyrosine	6·34	2·77	23·4	6·96	3·39	24·7	2·50	4·16	6·66	1·61	1·57
	0·48	0·23	1·53	0·46	0·28	1·55	0·61	1·00	0·76	0·33	0·15
		<0·05					<0·02	<0·01			<0·02
6-Hydroxydopamine	7·32	3·75	25·7	6·88	3·13	26·3	2·68	3·92	7·29	1·16	1·83
	0·59	0·47	1·7	0·49	0·24	1·46	0·50	0·69	0·81	0·12	0·12
							<0·05	<0·01		<0·05	<0·05
Reserpine	3·63	1·18	17·1	6·72	3·59	24·6	5·50	4·76	16·2	1·77	0·40
	0·77	0·36	2·57	1·00	0·44	2·72	0·91	0·59	4·89	0·23	0·40
	<0·001	<0·001						<0·05	<0·01		

The dose per snail of each drug was as follows: *p*-chlorophenylalanine, 5 mg, 48 h before experiment; hemicholinium, 5 mg, 16 h before experiment; L-3-4-dihydroxyphenylalanine, 5-hydroxytryptophan, α-methyl-3-4-dihydroxyphenylalanine, α-methyl-*p*-tyrosine, α-methyl-5-hydroxy-tryptophan, 200 μg, 1 h before experiment; 6-hydroxydopamine, 3 doses of 350 μg (in ascorbic acid) given at 24 h intervals, 14 days before experiment; reserpine, 3 doses of 350 μg given at 24 h intervals, 24 h before experiment. Control represents the mean of twenty experiments, drug values represent the mean of seven experiments. Only significant *P* values are included.

of the 5-HT epsp. L-3-4-Dihydroxyphenylalanine increased the initial and final heights of the ipsp and the ILD. 5-Hydroxytryptophan increased the number of stimuli required to reach the final 5-HT height.

This work presents further evidence for the identity of chemical transmitters in the snail brain.

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Actions of phenelzine on the interactions of the metabolism of tryptophan and dopamine in brain

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The acid metabolites 5-hydroxyindole-3-ylacetic acid (5-HIAA) and homovanillic acid (HVA) in cerebrospinal fluid (CSF) have been shown to be derived only from the metabolism of their parent amines, 5-HT and dopamine, occurring in brain. Recently (Moir, 1969) it was shown in dogs that an intravenous injection (50 mg/kg) followed by an intravenous infusion [(20 mg/kg)/h for 4 h] of L-tryptophan led to steady-state levels of the 5-hydroxyindoles in brain in which the concentrations of the 5-hydroxyindoles in various regions were all raised to between 2 and 3 times their normal control values. The concentrations of 5-HIAA in CSF from the cisterna magna during the 3–4 h period after the start of the tryptophan infusion were also three times control values. Surprisingly, concentrations of HVA in these same samples of CSF showed an even greater rise, being 14 times the normal control values; however, normal concentrations of dopamine and its main metabolites were found in the caudate nucleus. In the present experiments, dogs were pre-treated for 10 days with phenelzine (2 mg/kg subcutaneously) and then given, as previously described (Moir, 1969), an intravenous injection—infusion of L-tryptophan or an equivalent volume of saline.

The pattern of concentration changes of the amino-acids tryptophan and tyrosine in erythrocytes, CSF and brain following tryptophan or saline infusion were not affected by phenelzine. In saline-infused dogs phenelzine pre-treatment caused a fall in 5-HIAA concentration in CSF from the lateral ventricle, but not the cisterna magna, while in brain concentrations of 5-HT rose to 400% and 5-HIAA to 150% of normal.

In the phenelzine pre-treated animals a subsequent infusion of tryptophan did not increase brain 5-HT concentrations any further, and while brain concentrations of 5-HIAA showed a slight increase, its concentrations in CSF during the tryptophan infusion behaved exactly as during the saline infusion. These and earlier (Ashcroft, Crawford, Dow & Moir, 1969) results show that phenelzine inhibits the cerebral 5-hydroxyindole pathway at four separate points; tryptophan 5-hydroxylase, monoamine oxidase, 5-HIAA efflux from brain and 5-HIAA efflux from CSF.

The caudate nuclei of dogs treated with phenelzine alone showed high concentrations of dopamine and 3-methoxydopamine and low concentrations of 3,4-dihydroxyphenylacetic acid and HVA. The HVA was also in very low concentrations in CSF from the lateral ventricle and cisterna magna. In the dogs given subsequent