Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *86*, Suppl. II, 173-175, 1991

DEVELOPMENT OF NATURAL PRODUCTS AS DRUGS ACTING ON CENTRAL NERVOUS SYSTEM

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We have recently studied several natural product constituents which have effects on the CNS. (1) Tetrahydropalmatine (THP) and its analogues were isolated from Corydalis ambigua *and various species of* Stephania. *(+)–THP and (–)–THP possess not only analgesic activity, but also exert sedative-tranquillizing and hypnotic actions. Results of receptor binding assay and their pre- and post-synaptic effects on dopaminergic system indicate that (–)–THP and (–)–stepholidine are dopamine receptor antagonists while (+)–THP is a selective dopamine depletor. (2) 3–Acetylaconitine (AAC) is an alkaloid isolated from* Aconitum flavum. *The relative potency of analgesic action of AAC was 5.1-35.6 and 1250-3912 times that of morphine and aspirin, respectively. The analgesic effect of AAC was not antagonized by naloxone, but was eliminated by reserpine. In monkeys, after AAC was injected for 92 days, no abstinence syndrome was seen after sudden AAC withdrawal or when challenged with nalorphine. (3) Huperzine A (Hup-A) is an alkaloid isolated from* Huperzia serrata *which was found to be a selective ChE inhibitor and could improve learning and retrieval processes. Preliminary clinical studies showed that Hup-A improve short- and long-term memory in patients of cerebral arteriosclerosis with memory impairment. (4) Ranamargarin is a new tetradecapeptide isolated from the skin of the Chinese frog* Rana margaratae. *This peptide may mainly act on NK-1 receptor.*

Key words: tetrahydroprotoberberines – 3-acetylaconitine – huperzine A – ranamargarin – analgesia – dopamine receptor antagonists – cholinesterase inhibitors

The traditional Chinese medical practice and traditional Chinese drugs have a long history. Plants and animal materials are still being used in the traditional Chinese medical practice. In recent years, numerous studies have been done on these natural products in China. Using natural materials as a starting point scientists in our institute have attempted to isolate the active constituents and study their pharmacological effects. This paper presents some pharmacological aspects of the recent work in our Department on traditional Chinese drugs affecting the Central Nervous System (CNS).

1. TETRAHYDROPALMATINE (THP) AND ITS ANALOGUES

THP is the main active principle of *Corydalis ambigua*, a Chinese herb used as a traditional analgesic. Although it was isolated in 1929, its biological activities were not systematically studied until 1960's. Hsu & Kin (1962) and Kin et al. (1964) found that (+)– THP and (–) –THP possess not only analgesic activity but also exert remarkable sedative-tranquillizing and hypnotic actions. Since 1964, a number of THP analogs have been isolated from various species of stephania. These include (±)–tetrahydroprotoberberines (THB), (–)–stepholidine (SPD), (–)–scoulerine, (–)–THP, (–)–corydalmine, and (±)–corypalmine. They are all share a naloxone-resistant analgesic effect and have no affinity for opioid receptors, For example, iv injection of (–)–THP at 20 mg/kg to rabbits produced a marked analgesic, but (+)–THP at the same or even larger doses did not exhibit analgesic effect. (–)–THP or (±)–THP (40-50 mg/kg) significantly potentiated hexobarbital narcosis and reduced spontaneous activity in mice, and antagonized amphetamine induced *hyperactivity* and toxicity in aggregating mice, while (+)–THP exerted little effects. (–)–THP at 15 mg/kg potentiated the subthreshold dose of pentobarbital to induce sleep. Among the THPBs, (–)–THP is the first one to be listed as analgesic in Chinese Pharmacopoeia. Further studies demonstrated that (–)–THPBs are a new class of dopamine (DA) receptor antagonists. THPBs inhibited both [^{3}H] SCH 23390 and [^{3}H] spiperone binding suggesting that (–)–THPBs were mixed DA receptor antagonists. But (–)–THPBs were

more potent at the D_1 receptors. (−)−SPD for D_1 receptors was about 4-7 times more potent than that for D_2 receptor. In addition, (−) −SPD was 18 times more potent than haloperidol on D_1 receptors but 14 times weaker on D_2 receptors. (−)−THPB inhibited DA-stimulated adenylate cyclase activity supporting the notion that (−)−THPBs mainly act on D_1 receptors. The structure-activity studies of (−)−THPBs displayed the critical role of the OH groups on C_2, C_3, C_9 and C_{10}. The hydroxy group on C_2 is particularly important for high potency as in the case of (−)−SPD. In rotational experiments (Shi et al., 1984), THB, (−)−THP, (−)−corydalmine and (±)−corypalmine antagonized both rotations induced by apomorphine and amphetamine showing that THP and its analogs to possess antagonistic effects on DA receptors. (−)−SPD, on the other hand, exhibited an apomorphine-like effect which induced rats to rotate towards the unlesioned side. But (−)−SPD antagonized the amphetamine-induced turning effect towards the lesioned side. The results suggested that (−) −SPD had mixed partial agonistic properties. (−)−THPBs also antagonized stereotypy induced by apomorphine and produced catalepsy. All these results demonstrated that (−)THPBs were dopamine receptor antagonists. In traditional Chinese medical practice, *Corydalis ambigua* is an important analgesic. Pharmacological studies show that the analgesic effect of its active constituents (−)−THPBs was not blocked by naloxone suggesting that opioid receptors were not involved. Further studies are needed to understand their analgesic mechanism. Recently (−)−SPD has been used in the treatment of patients with brain DA dysfunction or migraine (Le et al., 1987). Further clinical studies are in progress.

2. 3-ACETYLACONITINE

The root of *Aconitum flavum* has been used as an analgesic in Ning Xia Autonomous Region. Two alkaloids I and II were identified as 3-acetylaconitine (AAC) and aconitine respectively. AAC was discovered in nature for the first time. The analgesic effect of AAc was studied by Tang et al. When mice writhing test, mice hot plate test, formalin test and rat tail-flick test were used, Tang et al. (1986a) found that the relative analgesic potency of AAC was 5.1-35.6 and 1250-3912 times that of morphine and aspirin respectively. Daily sc of AAC 0.25 mg/kg for 9 days in mice did not induce tolerance. In nalorphine-challenged test no jumping was seen in mice treated with AAC 3.35 mg/kg. When monkeys were given AAC sc in similar doses for 92-95 days, no abstinence syndrome was seen after sudden AAC withdrawal or when challenged with nalorphine 4 mg/kg on day 21, 41, 52, 62 and 92 respectivelly. In morphine-dependent monkeys, AAC did not suppress the abstinence syndrome evoked by sudden morphine withdrawal or by sc nalorphine 0.5 mg/kg. These results demonstrated that AAC was an non-narcotic analgesic. Lu et al. (1988) found that AAC-induced analgesia was markedly reduced by reserpine, dl-p-chlorophenylalamine, p-chloroamphetamine, 6-hydroxdopamine, dopamine, apomorphine or selegiline. The analgesic effect of AAC was enhanced by 5-HT, norepinephrine, cyclic AMP, pargyline or haloperidol. The results suggest that the analgesic effect of AAC is closely related to the CNS.

3. HUPERZINE A (HUP-A)

Huperzia serrata is used as anti-inflammatory agent in traditional medical practice and usually is accompanied with cholinergic side effects. Hup-A is an alkaloid isolated from *Huperzia serrata*. Wang et al. (1986) found that Hup-A exerted a potent inhibitory effect on ChE. The PI_{50} of Hup-A towards erythrocyte membrane and caudate nuclei AChE were 7.2 and 7.9 respectively. The inhibitory effect of Hup-A was about 3 times more than that of physostigmine with AChE, but less than that of physostigmine when tested with serum ChE (BuChE), thus, Hup-A exhibited a higher selectivity towards the true ChE. It has been reported that physostigmine improved learning and retrieval processes. The effects of Hup-A on learning and retrieval process of discrimination performance in rats were studied by Tang et al. (1986b) and Lu et al. (1988). In their experiments, rats were placed on a electrified grid in a Y-maze and trained to run into the light arm (safe area). The criterion of learning or retrieval was met after they had chosen the light arm 10 trials in succession. Hup-A injected ip before training caused a significant decrease in the number of trials to criterion. Facilitation of retrieval was also produced dose-dependently (36-167 μg/kg ip). Scopolamine, atropine or hemicholinum antagonized the effects of Hup-A on retrieval process. The results suggested that the facilitation actions of Hup-A were due to an effect on the central cholinergic system. Preliminary clinical studies

showed that Hup-A did improve short- and long-term memory in patients of cerebral arteriosclerosis with memory impairment.

4. RANAMARGARIN

Ever since the discovery of enkephalins by Hughes et al. (1975), several laboratories in China have been working on the involvement of endogenous opioid peptides in acupuncture analgesia. Tsou et al. (1987 for review) have been studying the role of enkephalins in acupuncture analgesia. Recently amphibian skin peptides have aroused a general interest because these peptides have their counterparts in mammalian gut and nervous systems. Erspamer et al. (1983) have undertaken a systematic biological and chromatographic screening of skin extracts of amphilians from most parts of the world. In search of new active peptides in the amphibian skin and in mammalian tissues, our laboratory together with the chemists in the Institute have carried out research on frog skin peptides since 1983 and several new neuropeptides have been discovered. Ranamargarin is one of them. The sequence of the peptide is Asp-Asp-Ala-Ser-Asp-Arg-Ala-Lys-Lys-Phe-Try-Gly-Leu-Met-NH_2 (Tang et al., 1989) and the structure has been confirmed by synthesis. The peptide is the largest among the amphibian tachykinins and its *N*-terminal amino acids are quite different from those of the other tachykinins. Ranamargarin produced fast and strong contraction of the longitudinal muscle myenteric plexus preparation of the guinea pig ileum. When the muscle was pretreated with a substance P antagonist, [*D*-Arg, *D*-Phe, *D*-Trp, Leu]-substance P, the effect of ranamargarin was blocked. Ranamargarin also produced a prominent hypotension effect when injected ivin rats. These results are in accordance with the tachykinin activity.

Although numerous studies have been done on several natural products in our Department, most of them are still in the initial stages. However, the results demonstrate that the natural products are a promising realm that awaits neuropharmacologists to exploit.

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